VOLUME 10 NUMBER 6

JUNE, 1920

Phytopathology

An International Journal

Official Organ of the American Phytopathological Society

CONTENTS

Physiological strains of Rhizoctonia solani ..... Jessie Booth 533

Relation of species of Pythium to the root-rot disease of sugar cane. C. W. Edgerton, E. C. Tims, and P. J. Mills 543

Plot arrangement to distribute neighbor influence in field trials. Jacob Hanley 563

Dendrophthora intoo-sanguinea Rehm on western white pine, Pinus monticola Dougl. ..... C. R. Stillinger 575

Mycorrhizas of Lobelia ..... A. S. C. Tison 585

Rust spots of maize, Ustilaginoidea ..... R. J. Haskell and W. W. Diehl 589

A method of testing the keeping quality of certain small fruits. 593

Interesting fungi found in the United States. ..... Caroline T. Rumbold 597

Resistance anthracnose of raspberry ..... H. S. M. Fellows 601

Phytopathological Notes 605

Book Review 609

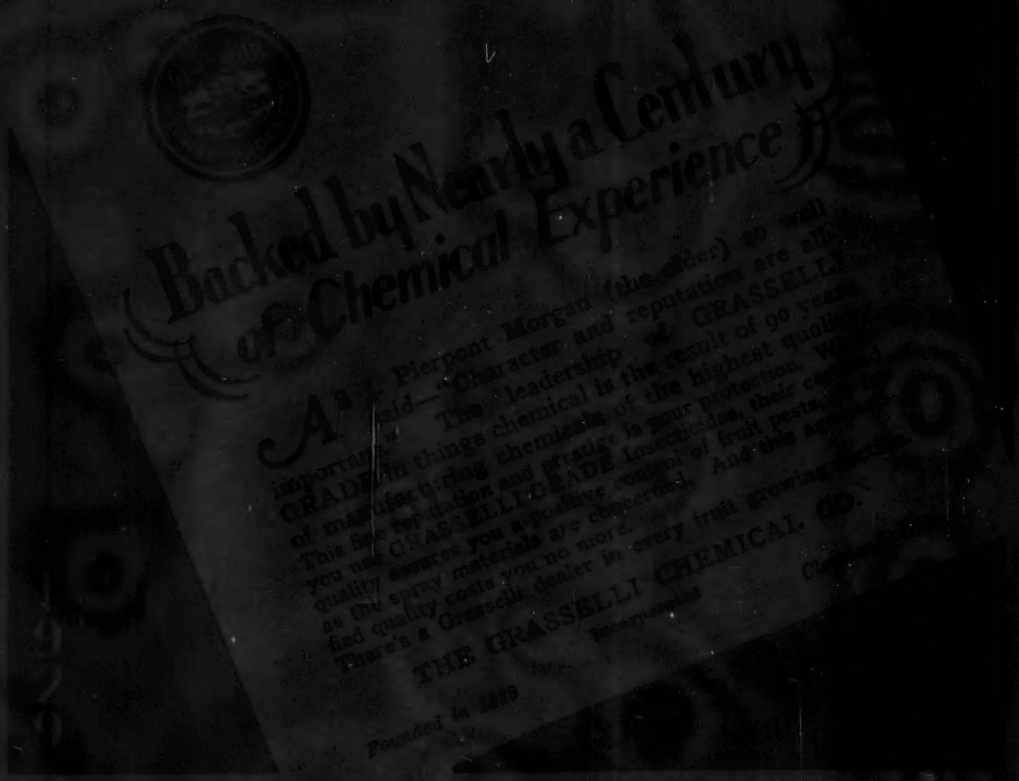
Price { $2.00 per year, United States and possessions, Mexico and Cuba
$2.25 per year, Canada
$3.00 per year, elsewhere

PUBLISHED MONTHLY BY THE SOCIETY

OFFICE OF PUBLICATION

Ore. Press Inn Orange Street, Lancaster, Pa.

Entered at the postoffice in Lancaster, Pa., as second-class matter.



UNITED STATES DISTRIBUTORS of

POTASH SALTS OF ALL GRADES

N. V. POTASH EXPORT MY.

25 West 45th St. NEW YORK	Hurt Building ATLANTA	Lamar Life Bldg. JACKSON, Miss.
McCormick Bldg. CHICAGO	First Nat'l Bank Bldg. BALTIMORE	Bank of San Jose Bldg. SAN JOSE

NV

Purchase from our advertisers. Mention Phytopathology when ordering.

PHYTOPATHOLOGY

VOLUME 19 NUMBER 6

JUNE, 1929

PHYSIOLOGICAL STRAINS OF *ALTERNARIA SOLANI*[1]

REINER BONDE

INTRODUCTION

It has been shown in previous publications that *Alternaria solani* (E. & M.) J. & G. can produce a tuber rot of potatoes (*Solanum tuberosum* L.) (4, p. 78; 5; 6; 7, p. 6–10; 8; 9) and that the fungus upon its isolation from potato-tuber lesions displays certain differences indicative of the occurrence of more than one strain (1; 5, p. 282; 6). The purpose of this publication is to describe these differences more fully and to discuss some strains which originated (*in vitro*) through saltation in culture. The strains are compared as to spore dimensions, pathogenicity on leaves and tubers, and, chiefly, pigmentation of the potato-agar substratum. Such pigmentation by the fungus in question is referred to by Rands (15, p. 20) but he did not study it particularly.

SOURCES AND VARIABILITY OF THE STRAINS USED[2]

The sources and variability of certain strains, chosen for more careful comparisons, are indicated as follows:

Strain C-1 was isolated in 1924 from a lesion on a Spaulding Rose tuber grown in Maine and shipped to Florida. It has remained stable.

Strain C-2 was obtained as for C-1 except that the shipper and stock were different. It produced a saltation (C-5) distinguished by a darker surface color.

Strain C-3 was isolated in 1924 from an Early Ohio tuber grown in Minnesota and sent to Maine for diagnosis. Saltations distinguished by darker surface color have originated from this strain.

[1] Most of this article is included in a thesis accepted in partial fulfillment of the requirements for the degree of Master of Science at the University of Maine. The writer wishes to make grateful acknowledgment to Dr. W. J. Morse, Dr. W. H. Eyster, and Dr. Donald Folsom, for advice given during the course of this study, and to Dr. Folsom for criticism of the manuscript. It is realized that the scope of these data is limited, but it has not been feasible to supplement them by further studies so that it seems desirable to report the available results without further delay.

[2] The letters C, I, and N, here designate respectively chromogenic, intermediate or variable, and nonchromogenic strains.

Strain C-4 was isolated in 1922 from Spaulding Rose stock grown in central Maine. It has remained stable.

Strain C-5 arose as a saltation (1925) in a culture of strain C-2 and has remained stable.

Strain I-1 was isolated from the same stock as C-4 but from a different lesion. It varies in culture from slightly chromogenic to nonchromogenic, generally coloring the medium pink instead of a deep red or carmine.

Strain I-2 originated as C-4 and I-1 but from a different lesion. It is variable on potato agar and may range from nonchromogenic to chromogenic, producing a distinct carmine.

Strain I-3 originated in 1925 as a saltation in a culture of N-2 and varies in chromogenicity.

Strain N-1 was isolated in 1924 from the same stock as C-2 but from a different tuber. It produces saltations with a more rapid growth rate, which are sometimes slightly chromogenic.

Strain N-2 was isolated in 1922 from the same stock as C-4, I-1, and I-2 but from a different lesion. It produced saltation I-3.

Strain N-3 arose in 1925 as a saltation in a culture of I-2 and has remained stable.

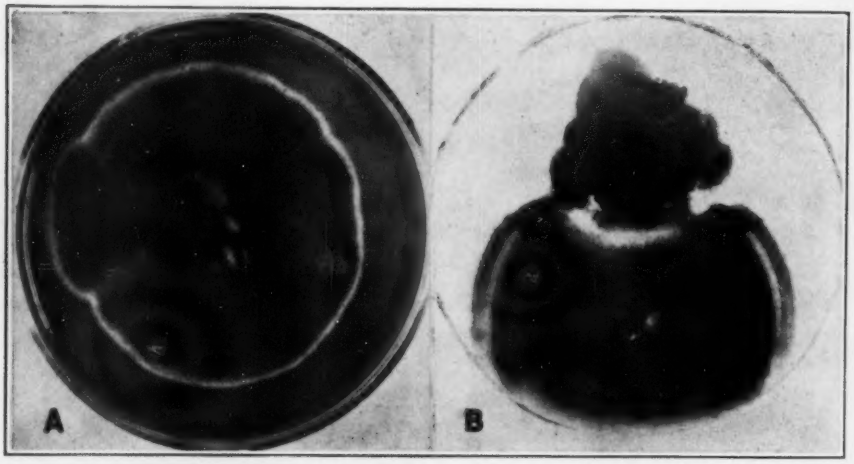


FIG. 1. Typical saltations of *Alternaria solani*. A. A saltation appearing as a darker and faster-growing sector. B. The lower colony differs from the upper (original strain) in that it is slightly chromogenic and grows more rapidly on artificial media.

Typical saltations are shown in figure 1. A is a chromogenic culture of strain I-3 with a saltation appearing as a sector that is darker and faster growing than the main body of mycelium. Often such sectors differ from their parent culture by being nonchromogenic. B is the result of two trans-

fers made thirteen days previously from the same tube culture of strain N-1. The smaller colony is typical of the strain. The other colony differs from the original culture by being slightly chromogenic and producing a faster growth on media.

The saltation history in one related group of cultures is shown in figure 2. It is noted that the chromogenic strain produced both nonchromogenic and intermediate forms similar to those isolated from nature. One strain obtained from this source as a saltation has remained nonchromogenic and is only slightly pathogenic.

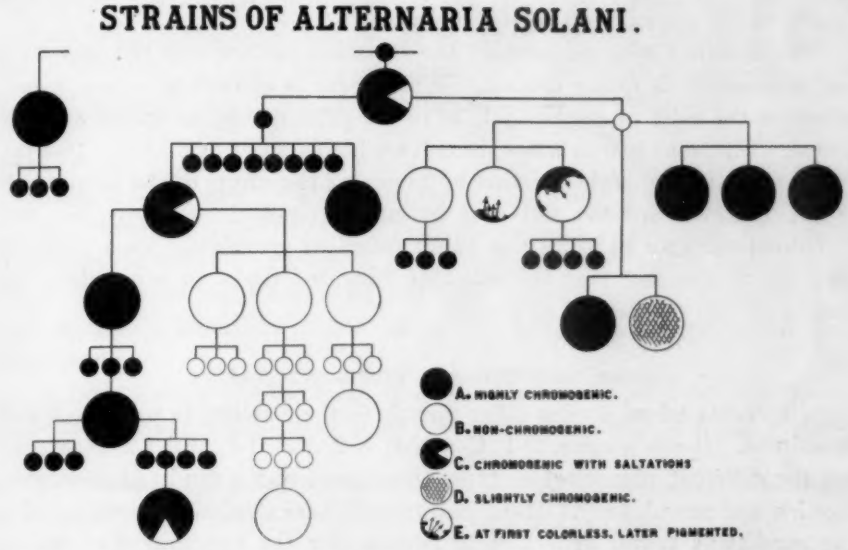


FIG. 2. Diagram showing chromogenicity in progeny originating from a single chromogenic culture of *Alternaria solani*. Small circles indicate test-tube cultures and large circles indicate plate cultures. The latter supply all the saltant sectors but note that transferring either from tube to plate or from plate to tube may be followed by the appearance of a change in chromogenicity.

INDUCING SPORE PRODUCTION IN CULTURE

Spore production on media was found rather difficult to induce and is in need of further study. An examination of numerous cultures grown on potato agar has disclosed only an occasional spore. Cultures grown on prune agar and on corn agar failed to produce spores. Two strains were cultured on sterilized potato plugs, on which spores were more common but were by no means abundant. These spores were deformed in most cases, having swollen cells and crooked or twisted beaks. (Normal spores are depicted in PHYTOPATHOLOGY, Vol. 15, Plate V.) (5.)

As shown by Rands (16) and Kunkel (12), the most successful method of spore production of *Alternaria solani* involves the laceration and desiccation of agar cultures. Rands grew the cultures on petri dishes of hard potato agar and after 10–12 days they were shredded to bits and evenly distributed over the surface of the dishes. The humidity must be controlled for 24 to 48 hours in such a manner as to allow partial desiccation, but not complete drying or hardening of the cut surfaces. Such moisture control is most readily secured by removing the lid and exposing the dish to sunlight in a moist chamber and by atomizing the cultures with sterile water if too dry. If the procedure is successful, spores will develop in great profusion on the cut surfaces of the medium.

The moisture relation between the shredded culture and the surrounding atmosphere is rather critical. When there is enough moisture to condense on the sides of the bell-jar, or in the petri dishes, no spores are produced. The same is true when the culture becomes dry and hard. One can regulate the atmospheric moisture by removing the covers of the petri dishes if the cultures are too wet and by atomizing with water if too dry. Shredded cultures in large test tubes failed to sporulate. This probably was due to the fact that the moisture liberated made an atmosphere too humid for the production of spores.

SPORE PRODUCTION BY DIFFERENT STRAINS

Alternaria solani strains differ greatly in their ability to produce spores in culture. Seven strains, C-1, C-3, N-1, N-2, N-3, I-1, and I-2, representing the different physiological types, were used in the study of spore production and morphology. Many cultures of these strains were subjected to the conditions found generally to be best for the production of spores. Some strains sporulated in great profusion, while others either failed to sporulate or did so only meagerly. Many attempts to produce spores with the latter strains have been made but the results were similar, in that no spores or relatively few appeared. It was found that the strains vary greatly in their ability to fruit on an artificial medium such as potato agar. Strains C-1, C-3, N-2, and I-1 were consistently good spore producers. Strains N-1, N-3, and I-2 have in all cases failed to sporulate freely.

The degree of chromogenicity of a strain is not correlated with its ability to produce spores in culture. Good or poor spore formation was found associated with either chromogenic or nonchromogenic strains. Likewise both chromogenic and nonchromogenic strains are included among those which have failed entirely to form spores on artificial media.

The *Alternaria solani* strains isolated by the writer differ as to the kind of mycelial growth. A black-surface, deep, and compact mycelial mat is

associated with abundant spore production. Strains or cultures possessing a white-surface mycelium, thin and loose in texture, are poor spore formers. There is evidence also that during the process of continued culture some strains have lost their power of forming spores.

BIOMETRY OF SPORE DIMENSIONS OF DIFFERENT STRAINS

The different strains used in the study of spore dimensions were grown on potato agar and made to sporulate in the manner previously described. The medium and environmental factors were made as uniform as possible for all cultures. The writer, however, found it very difficult to control the environment of the mycelium in the culture tubes and differences in the environment may explain the great variation in the mean dimensions found to exist in different cultures of the same strain, to be noted later.

Some strains, such as I-2, produced spores very sparsely and therefore were treated many times before enough spores were produced from which to obtain measurements. Consequently their spores were not all grown at the same time nor were they taken from the same lot of medium. Attempts were made to measure at least 50 spores chosen at random for each population. The spores usually were washed from the cultures with distilled water and mounted immediately on slides for microscopic observation and measurement.

In table 1 are given the mean spore dimensions for cultures of different *Alternaria solani* strains with their constants. A study of table 1 brings

TABLE 1.—*Dimensions of spores from strains of Alternaria solani grown on potato agar*

Strain	Culture Number	Number of spores measured	Length (microns)		Width (microns)	
			Mean and P. E.	S. D. and P. E.	Mean and P. E.	S. D. and P. E.
C-1	1	50	123.80 ± 4.56	47.78 ± 3.22	14.50 ± .200	2.10 ± .142
	2	47	148.19 ± 3.74	39.25 ± 2.65	16.24 ± .387	3.90 ± .274
	3	50	163.40 ± 3.62	37.92 ± 2.56	17.26 ± .393	4.12 ± .278
	4	100[a]	142.70 ± 2.46	36.44 ± 1.74	15.25 ± .163	2.42 ± .115
C-3	1	50	122.00 ± 3.03	31.82 ± 2.15	15.40 ± .227	2.38 ± .160
N-1	1	41	140.36 ± 3.27	31.09 ± 2.31	15.44 ± .237	2.25 ± .167
N-2	1	100[a]	150.40 ± 2.79	41.43 ± 1.97	16.59 ± .169	2.51 ± .119
N-3	1, 2, 3	50	134.00 ± 3.08	32.32 ± 2.18	14.74 ± .187	1.98 ± .133
I-1	1	50	137.40 ± 3.20	33.51 ± 2.26	14.24 ± .166	1.74 ± .117
	2	48	143.90 ± 3.37	34.64 ± 2.38	14.60 ± .202	2.08 ± .142
	3	50	157.00 ± 2.97	31.11 ± 2.10	15.38 ± .190	1.99 ± .134
I-2	1	7[b]	119.30 ± 6.78	26.60 ± 4.79	14.57 ± .405	1.59 ± .028

[a] Preserved in 4-per cent solution of formaldehyde between sporulation and measurement.

[b] From several cultures; many cultures of this strain examined with no spores to be found.

out the fact that the spore dimensions vary greatly for different cultures of the same strain. Culture 1, strain C-1, gave spores that were among the shortest ones measured. Culture 2 from the same strain produced spores having the greatest mean length. Here exists as great a variation as is found by comparing any two distinct strains.

The mean widths of the spores from different strains ranged from 14.24 μ for culture 1, strain I-1, to 17.26 μ for culture 3, strain C-1. The variation between the mean widths of spores from cultures of strain C-1 was nearly as great as the extreme difference between the means of different strains (14.50 ± .200 for culture 1 to 17.26 ± .393 for culture 3 of strain C-1). A greater spore width was generally associated with a greater spore length for the strains measured. In general, the variation of the width was less than that of the length.

No one strain was consistently wider or longer than the others. Apparently, uncontrolled factors caused a greater variation in mean length and width than did a difference in strain. It is probable that some strains are inherently different as to spore dimensions, but with the present methods they can not be differentiated.

GENERAL NATURE OF PIGMENTATION BY ALTERNARIA SOLANI

Unless otherwise specified, "pigmentation" in this paper means the coloration of the agar substratum by products of fungal growth and is to be distinguished from pigmentation of the mycelium which is the subject of most of the literature dealing with the color of fungi *in vitro*.

On potato agar[3] a typical young chromogenic culture at first causes a clear yellow pigmentation which spreads in advance of the mycelial growth of the fungus. This color changes in time to a deep brick red or carmine.[4] It would seem that the *Alternaria solani* strains cultured by Rands were all of a chromogenic type. He also states that "after 7 to 10 generations in pure culture the pigmentation is much diminished and in some cases has been observed to almost disappear" (15, p. 20). However, cultures observed by the writer and his associates in Maine showed marked differences in chromogenicity directly after isolation from potato tubers. About nine-tenths of the several hundred cultures thus obtained were highly chromogenic. The others were either nonchromogenic, some remaining so under all conditions to which they were subjected, or they ranged from nonchromogenicity to marked coloration. Chromogenicity could not be predicted from the appearance of the tuber lesion.

[3] The potato agar used in these studies was made up of 200 grams of pared potato tuber, 20 grams of dextrose, 30 grams of agar, and 1 liter of distilled water. It was slightly acid with a pH value of approximately 6.7–6.8, using brom thymol blue as the indicator.

[4] Color terminology here is based upon Ridgway (17).

Very little is known regarding the chemical nature of the pigment of *Alternaria solani*. When the carmine agar of old chromogenic cultures is shredded and washed with water, a solution of the pigment is obtained which is orange or yellow when made acid and carmine when made alkaline. Therefore the pigment is an indicator. These data also suggest that the fungus increases the alkalinity of potato agar, which will be proved later.

EFFECT OF MEDIUM ON PIGMENTATION

Rands (15, p. 20) reports that *Alternaria solani* produces no coloration when grown on acid casein agar, nutrient gelatin containing dextrose, starch nitrate agar, and cellulose agar. The effect of difference in the kind of medium was brought out when a series of isolations made on prune agar from potato lesions gave in all cases nonchromogenic cultures. Different chromogenic strains of *Alternaria solani* grown on prune agar at different temperatures produced a slight discoloration approaching a light coffee brown or pale xanthine orange, while nonchromogenic forms caused merely a slight murkiness on the agar.

EFFECT OF TEMPERATURE ON PIGMENTATION

During the winter of 1924–1925 various strains were grown at different temperatures to test the influence of this environmental factor upon the degree of pigmentation. The following approximate mean temperatures were secured in dark chambers by the use of an electric device with automatic control together with ice: 5°, 10°, 15°, 20°, 25° and 30° C. The three lowest temperatures were known to fluctuate one or two degrees, varying most from the mean just before and after the process of icing which occurred about once a week.

The highly chromogenic strains continued to be chromogenic in the 20°, 25°, and 30° chambers. Strain C-1 was chromogenic under all conditions except at 5° C. Strains C-2 failed to produce pigment at temperatures below 20° C. The nonchromogenic strains N-1, N-2, and N-3 failed to produce pigmentation in all the cultures observed.

One variable strain, I-1, was slightly chromogenic at 25° and 30° C., producing faint pink or xanthine orange coloration, but at 5° to 20° C. it was nonchromogenic with an occasional trace of pigment as growth progressed. Strains I-2 and I-3 were nonchromogenic at 5° and 10° C. Chromogenic cultures of I-3 became nonchromogenic with age, a phenomenon also noted to occur in other chromogenic cultures.

These studies indicate finally that temperature influences the amount of pigment produced by *Alternaria solani* on potato agar. In general, temperatures below 15° C. may entirely inhibit the production of color. At

approximately 20°, retardation occurs, while at 25° and 30° a maximum amount of color is produced. The maximum rate of growth also took place in the 25° and 30° chambers. The formation of pigment is generally correlated with the rate of growth, although a luxuriant mycelium developed ultimately at the lower temperatures.

Subtransfers were made from the nonpigmented cultures of the chromogenic strains grown at the lower temperatures to test whether the condition was merely temporary due to environment or was permanent due to some change in the mycelium. In no case could it be noted that the ability to produce color had been permanently affected. The mycelium transferred from nonpigmented cultures of normally chromogenic strains consistently produced pigment when grown under higher temperatures. Although these transfers showed that low temperature had no permanent effect on the chromogenicity of a strain of the fungus, low temperature did have a permanent effect on the actual culture exposed. When nonpigmented cultures of chromogenic strains were removed to the chambers with higher temperatures, pigmentation was not produced. This would suggest either that the low-temperature cultures had developed an inhibiting principle in the substratum which prevented the production of the color, or that the pigmentation was a function of rapid growth and, therefore, impossible under any conditions after growth of the substratum had been completed.

EFFECT OF INITIAL HYDROGEN-ION CONCENTRATION ON PIGMENTATION

The variability in chromogenicity in cultures of *Alternaria solani* raised the question as to whether slight differences in acidity of the medium might be responsible for this phenomenon. Accordingly, transfers from vigorously growing cultures of both nonchromogenic and chromogenic strains of the fungus were made to media adjusted to different degrees of acidity and alkalinity.

For these studies strain C-1 and chromogenic cultures of strain I-2 were taken as representatives of the more highly chromogenic types, strain I-1 as representative variable form, and N-3 as a consistently nonchromogenic strain.

A preliminary test was made to determine whether a critical point, at which chromogenicity was affected, could be approximated. Different numbers of drops of N/20 HCl and N/20 NaOH were added to tubes each containing 10 cc. of slightly acid potato agar, following the "drop method" suggested by Hopkins (10, 11). Ten different concentrations were thus obtained ranging from distinctly acid to distinctly basic. The cultures were grown in duplicate at 25° and 30° C. and observations were made at five- to ten-day intervals. A third set of noninoculated tubes, representative

of all degrees of acidity or alkalinity, was kept for a later determination of the pH value.

The distinctly chromogenic strains were not greatly influenced by addition of acid or base. The nonchromogenic strain N-3 failed to produce pigmentation. In the tubes of the two highest acid concentrations, growth was slightly retarded and the pigment formed by the chromogenic strains was slightly subdued and more yellow. The variable strain I-1, on the other hand, was greatly affected by initial acidity and failed to produce pigment at the two highest acid concentrations. The less acid cultures were slightly pink and turned carmine with age. The color change was very striking at this point, the pH value of which was then determined. A set of colorimetric standards was made using various indicators (2, p. 80–81). Noninoculated check tubes, several weeks old, were melted with the indicators present in them and the results showed that the critical point for pigment production was between pH 4.6 and 5.6, within the range of methyl red. It should be emphasized that this critical point refers to the initial hydrogen-ion concentration at the time of inoculation and not at the time when pigmentation is effected. In the interval, as will be shown, the hydrogen-ion concentration is altered by the fungus.

The experiment was repeated and a series of tubes with a greater range of acidity was used. The results were similar to those of the first, with the exception that the variable strain (I-1) was nonchromogenic in all the tubes of media having a greater initial acidity than pH 4.2 and 4.0. An occasional noncolored culture occurred in tubes with a lower initial acidity. This strain was also grown on media made acid with N/5 instead of N/20 HCl, thus securing a still wider range of acidity. In this series noncolored cultures were consistently secured where the initial acidity of the medium was more than pH 4.2.

Similar sets of cultures of the same variable strain were made basic by adding N/5 NaOH to tubes containing 10 cc. of slightly acid agar. The basic cultures produced intense carmine pigmentation in most cases. The pigmentation faded when the NaOH was added in excess of 9 drops per 10 cc. The color of the less basic cultures approached that produced normally by chromogenic strains. As stated previously, a solution of the pigment from red cultures also became distinctly carmine when made basic.

These data are not in accordance with the observations made by Rands (15, p. 20), who apparently had still different strains of the fungus. He found that the fungus produced a red to deep red color on distinctly acid media (+20 Fuller's scale), a yellow color on slightly acid cultures and practically no pigment in alkaline cultures. In view of the observations already made, the writer believes that with more extensive study other

strains can be obtained which will differ greatly in their reactions to the hydrogen-ion factor.

EFFECT OF LIGHT ON PIGMENTATION

Cultures of the different strains were grown in the greenhouse in the presence of diffuse light and five successive attempts under these conditions did not affect the amount of pigmentation produced by a chromogenic strain. Nonchromogenic strains likewise were not affected visibly. Under greenhouse conditions the growth of some cultures was retarded and the pigment was made slightly dull.

Strain I-1, however, was influenced by light, and cultures grown in the greenhouse were constantly more pigmented than those in the dark chambers. Instead of being slightly pink or nonchromogenic they were yellow or xanthine orange. To verify further the effect of light on this variable strain, a more careful test was performed. Uniform tubes of agar were inoculated with a single-spore strain (from strain I-1) and lots of three tubes each were placed in the greenhouse and in the 20° dark chamber. The cultures in the dark failed to produce color, while those grown in diffuse light developed a reddening approximating the intensity observed in cultures of the more constantly red strains. This experiment was repeated several times with similar results.

Test-tubes containing the strain just previously referred to were wrapped in photographers' black paper and grown in the greenhouse along with similar tubes not wrapped. These two sets of cultures were compared with a third set of unwrapped cultures kept in the dark chamber. The cultures darkened by paper and those in the dark chamber produced a slight pinkish discoloration in contrast to the reddish brown and xanthine orange of the tubes kept in diffuse light.

EFFECT OF WATER CONTENT OF AGAR ON PIGMENTATION

Desiccation of the agar appeared not to influence the amount of pigmentation produced by the variable strain I-1. Tubes of neutral agar were desiccated by storage in a warm, dry place. To some of these tubes water was added. After being melted and reslanted both sets of tubes were inoculated and grown in the greenhouse either in the presence or in the absence of light. Similar cultures on desiccated and rewatered agar also were grown in the 20° C. dark chamber in a series parallel with that in the greenhouse. Desiccation in this test did not affect the degree of pigmentation. The cultures in both desiccated and moist media were only slightly colored in the chamber and in the tubes darkened by the black paper. In contrast, all of those kept in diffuse light developed the xanthine orange pigmentation.

INFLUENCE OF ALTERNARIA SOLANI ON THE HYDROGEN-ION CONCENTRATION OF POTATO AGAR

As stated in the foregoing, a typical culture produces an intense red color in potato agar. By treating a shredded culture with water or grain alcohol, this pigment is readily removed. As observed above, it was found that the pigment has the properties of an indicator, and is dull orange or amber in acid solution and deep carmine or wine color in basic solution.

In view of this phenomenon and the fact that cultures usually become more highly pigmented with age, an attempt was made to determine the influence of the fungus on the hydrogen-ion concentration of the medium. A nonchromogenic strain, N-3, was grown on potato agar in the presence of different indicators. In the first trial brom phenol blue, methyl red, brom thymol blue, and phenol red were used (2, p. 80–81). The indicator was added to the melted agar and N/5 HCl or NaOH added until series of tubes were obtained having the proper pH ranges for the respective indicators. The tubes were then slanted and inoculated. By growing the fungus in the presence of these indicators and observing the color changes, it was expected that a clue could be obtained as to the amount of acidity or alkalinity produced in the medium.

The growth of the fungus destroyed the efficiency of brom phenol blue, brom thymol blue, and methyl red. That is, the media lost the distinctive colors produced by these indicators and became non-pigmented. Phenol red, however, was not inactivated. The medium originally was slightly acid, having a pH value of approximately 6.6 to 6.8. In the presence of phenol red the medium supporting the growth of the strain in question became distinctly red in all cases, demonstrating that alkalinity had been produced beyond about pH 8.0. The experiment was repeated with the same nonchromogenic strain, which again produced an alkaline reaction as indicated by the phenol red.

The chromogenic strains were found to be unsuitable for study by this method because the pigment they produce is intense enough to mask the colors of the indicators. Therefore the culture-washing method was again resorted to with cultures of several nonchromogenic and chromogenic strains. In dilutions thus obtained the natural indicator (*i.e.*, the pigment produced by the fungus) was not concentrated enough to mask the phenol red, which gave a color reaction at the basic end of its range. Phenolphthalein, however, gave an acid color reaction showing the pH value of the solution to be less than 8.3–8.4.

The cultures of the preceding test were from 14 to 60 days old. It was thought probable that after a longer period of growth the pH value might be higher. Therefore cultures were grown for several months in the presence of phenolphthalein. In that time the alkalinity produced was not

great enough to bring about the red color in the indicator. The addition then of NaOH gave the basic reaction and showed that the indicator was still present and active.

THE PIGMENT AS AN INDICATOR

The above data throw some light on the nature of the chromogenicity of the different strains. The degree of color is dependent on two factors, namely, the amount of natural indicator and the alkalinity produced by the organism in the medium. The nonchromogenic strains increase the alkalinity like the other strains but do not produce this natural indicator.

The variable strain I-1 is slower to produce pigmentation but in old cultures sufficient amounts of natural indicator and alkalinity may occur to give a decided carmine hue. This may explain the reason why cultures, at first thought to be nonchromogenic, later become decidedly colored. Young 3- to 6-day-old cultures of the chromogenic strains are yellow because the medium is not yet alkaline enough to change the pigment to the carmine color. Plate cultures, carmine in color, have been made yellow by the addition of a weak acid. In such cases the fungus produced in a few days enough basic substances to neutralize the acid and the carmine color again appeared.

An attempt was made to determine the active pH range of the indicator produced by the organism. Water and alcoholic extracts of the indicator obtained from chromogenic cultures were tested for their color reaction in standard pH solutions for the range from 3 to 7.8. This range was found to be too acid to change the pigment of the fungus from yellow to red. This would indicate that a greater alkalinity than pH 7.8 is necessary to produce the red color of this fungous product. This confirms the preceding conclusions as to the pH value of old cultures.

PRODUCTION OF A TOXIC SUBSTANCE BY NONCHROMOGENIC STRAINS

There is evidence that the nonchromogenic strains produce a substance or substances toxic enough to inhibit both the growth and the pigmentation of the chromogenic strains. It was noted that a chromogenic strain planted in an agar plate on which a nonchromogenic strain was already growing often failed to grow. The mycelial mat then was removed from nonchromogenic cultures and the remaining agar melted and reslanted. This medium would not support the growth of the chromogenic strain C-1. The experiment was not extended to include the other strains.

When two chromogenic strains are grown simultaneously in the same plate of potato agar, the pigment readily diffuses throughout the medium. However, when the nonchromogenic strain N-3 was planted in close proximity to the chromogenic C-1 strain in the same plate, the pigmentation

of the agar was retarded in the region about the nonchromogenic organism. The chromogenic strain continued the formation of pigment in the part of the plate remote from the growth of the nonchromogenic strain. That the production of the natural indicator and not that of alkalinity is inhibited is evidenced by the alkalinity of nonchromogenic cultures.

PATHOGENICITY OF STRAINS ON LEAVES AND TUBERS

A study of the relative pathogenicity of the strains of *Alternaria solani* revealed that a great difference exists in their ability to infect potato foliage. In preliminary inoculations, however, the tested strains were equally virulent when tested on potato leaves. Two chromogenic strains, C-1 and C-4, and two nonchromogenic strains, N-1 and N-2, were used in this initial comparison. The four strains used in these tests were all pathogenic and caused rapid collapse when inoculated onto potato plants.

Clinton and McCormick (3) found moist petri dishes convenient chambers for the inoculation of leaves with certain rust fungi. The writer adopted a modification of their method for the study of the virulence of *Alternaria solani* strains. Damp chambers about 16 cm. in diameter were used for potato-leaf inoculations. Heavy blotting paper was cut to fit the bottom of each container. The blotter was moistened with water and marked with an indelible pencil into four equal sectors. Susceptibility depends somewhat on the age of the leaves. Accordingly, four similar leaflets from the same leaf, taken from a plant of the Green Mountain variety grown in the greenhouse, were placed in the four sectors to be respectively inoculated with four strains of the fungus. A fifth leaflet from the same leaf was placed in the middle, at the junction of the sectors, as a noninoculated control. A second leaf was used in the same way in each chamber. In each series of inoculations, strain C-1 was compared with three other strains. Each comparison was made on 30 leaves in 15 damp chambers.

When the mycelium had grown sufficiently to cover most of the surface of the medium in the tubes, inoculations were made by placing uniform bits of the mycelium on the ends of the leaflets. The rapidity with which the fungus destroys potato leaves depends to some extent on the prevailing temperature and moisture. Moisture was applied by means of an atomizer whenever the inoculum showed evidence of becoming too dry. The culture chambers were placed in diffuse sunlight in the greenhouse. Total darkness in controlled temperature chambers caused chlorosis and rapid collapse of the inoculated leaflets. Direct sunlight often resulted in a withering of the leaves and in desiccation of the inoculum.

The degree of pathogenicity was estimated by measuring the diameter of the brown, obviously infected area associated with the inoculum on the leaf surface, 5 or 6 days after inoculation. The diameter of the two spots

on each leaflet were averaged and the data analyzed by Student's method (13, 14). The means and the probability or odds that the difference is significant and not due to mere chance are given in table 2 for the two series of comparisons that were made.

The leaf inoculations with the various strains showed that a great variation in pathogenicity may exist. Strain C-1 which was used as the control was considerably and significantly more pathogenic than strains N-1, N-3, and I-1, while, according to the odds, it was in the same class with strains C-5 (dark) and N-2 and was not much more pathogenic than strain C-2. The last comparison is interesting because strain C-5 originated as a saltation from strain C-2, the saltation differing from its parent by possessing a darker colored mycelium.

TABLE 2.—*Comparison of strains of Alternaria solani as to pathogenicity on potato leaves in vitro*

Series[a]	Strains compared, with mean diameter of lesion (in mm.)		Significance of difference expressed in odds[b]
1	C–1 (17.43)	C–2 (14.17)	About 76.5
	C–1	C–5 (17.67)	Less than 2.37
	C–1	N–1 (3.60)	Over 9,999
	C–2 (14.17)	C–5 (17.67)	About 76.5
	C–2	N–1 (3.60)	Over 9,999
	C–5 (17.67)	N–1	Over 9,999
2	C–1 (11.87)	N–2 (11.70)	About 8.82
	C–1	N–3 (4.70)	Over 9,999
	C–1	I –1 (3.67)	Over 9,999
	N–2 (11.70)	N–3 (4.70)	Over 9,999
	N–2	I –1 (3.67)	Over 9,999
	N–3 (4.70)	I –1	About 5.90

[a] Each series consisted of 30 comparisons; see text, p. 545.
[b] Odds should be 30 or over to be significant.

The variation in the ability of different strains to infect foliage raised the question as to whether the strains might also show differences in virulence when inoculated into potato tubers. The strains used for the leaf-infection studies were grown on steam-sterilized wheat kernels. The wheat grains covered with the fungus were placed on freshly harvested Spaulding Rose tubers and incubated in moist chambers. Each tuber was inoculated with 10 kernels.

Strain I-1 was consistently and undoubtedly pathogenic in these tests. Strain I-3, originating as a saltant, was very weakly parasitic, causing only a few small and shallow lesions. All the other strains failed to produce spotting and lesions. It is significant that in a repetition of the test four substrains originating from single-spore colonies of strain I-1 were all pathogenic. The pathogenic strains were reisolated from the tuber lesions,

compared with the original cultures, and found to be identical with them.

It has seemed possible that strains of *Alternaria solani* might lose their virulence during continuous culturing on artificial media. However, strain I-1 has been cultured since 1922 and has not lost its pathogenicity to tubers. This would seem to show that loss of pathogenicity does not necessarily occur on artificial media within four years.

Strain I-1, though most pathogenic on tubers, was among the strains less pathogenic to potato leaves, while a reverse relation holds for some of the other strains.

These comparisons as to relative virulence indicate clearly that a great difference may exist between certain strains in their ability to attack potato foliage. Whether these differences exist in the field is an interesting question not yet answered. How often such differences arise through saltation, both in culture and in nature, is a problem awaiting attention. With a production of new strains in nature, the problem of early blight control might become more complicated than it seems to be at present. Various strains may be found to differ as to their pathogenicity to the varieties of the potato or genera and families of host plants. It may be proved eventually that under natural conditions some strains are virulent on potato foliage but unable to infect tubers.

SUMMARY

(1) *Alternaria solani* comprises strains which can be differentiated on the basis of the production of spores, the formation of pigment in potato agar, the rapidity of growth, the appearance of the mycelium in culture, the formation of lesions on detached leaves and tubers and frequency of saltations.

(2) Spore production required particular conditions. The strains varied as to the abundance of spore production under apparently similar conditions. When spores were produced their mean dimensions varied as much in different cultures of the same strain as in cultures of different strains, so that spore measurements did not serve to differentiate strains.

(3) The great majority of the strains isolated from lesions on potato tubers were chromogenic on potato agar, turning it red. Other strains were nonchromogenic, and some were intermediate or variable as to chromogenicity on potato agar. The pigment is an indicator which in aqueous or alcoholic solution turns from yellow (acid color) to deep red or carmine (alkaline color) at a pH value of about 7.8. Temperature influences the amount of pigment produced by certain more or less chromogenic strains, the temperature at the higher range of growth being optimum. In one intermediate strain the chromogenicity was inhibited by a lower initial hydrogen-ion concentration and by the absence of light but was not affected

by desiccation of the medium. Chromogenic strains do not redden prune agar. All strains increased the pH value of potato agar from about 6.6 to 8.3, as indicated by tests of aqueous and alcoholic washings of the agar. Nonchromogenic strains made potato agar toxic to chromogenic strains and prevented pigment formation by the latter.

(4) Inoculation by placing sterile mycelium on detached potato leaflets, or tubers, in damp chambers revealed highly significant differences in the pathogenicity of certain strains.

MAINE AGRICULTURAL EXPERIMENT STATION,
ORONO, MAINE.

LITERATURE CITED

1. BONDE, REINER. Variation of strains of *Alternaria solani* isolated from lesions on potato tubers (Abst.). Phytopath. **17**: 56. 1927.
2. CLARK, W. MANSFIELD. The determination of hydrogen ions. 480 pp. Edition II. Williams & Wilkins Co., Baltimore, 1925.
3. CLINTON, G. P., and FLORENCE A. MCCORMICK. Rust infection of leaves in Petri dishes. Connecticut Agr. Exp. Sta. Bul. 260: 475–501. 1924.
4. FLORIDA AGRICULTURAL EXPERIMENT STATION. Report for year 1925–26. 125 pp.
5. FOLSOM, DONALD, and REINER BONDE. *Alternaria solani* as a cause of tuber rot in potatoes. Phytopath. **15**: 282–286. 1925.
6. ————, ————. *Alternaria solani* as a cause of tuber rot in potatoes (Abst.). Phytopath. **15**: 49. 1925.
7. GRATZ, L. O. Irish potato disease investigations, 1924–25. (A preliminary report.) Fla. Agr. Exp. Sta. Bul. 176: 3–23. 1925.
8. ————, and REINER BONDE. Alternaria tuber rot of potatoes (Abst.). Phytopath. **16**: 68. 1926.
9. ————, ————. Infection of potato tubers by *Alternaria solani* in relation to storage conditions. Florida Agr. Exp. Sta. Bul. 187: 167–182. 1927.
10. HOPKINS, E. F. Hydrogen-ion concentration in its relation to wheat scab. Amer. Jour. Bot. **9**: 159–179. 1922.
11. ————. Note on the hydrogen-ion concentration of potato dextrose agar and a titration curve of this medium with lactic acid. Phytopath. **11**: 491–494. 1921.
12. KUNKEL, L. O. A method of obtaining abundant sporulation in cultures of *Macrosporium solani* E. & M. Mem. Brooklyn Bot. Gard. **1**: 306–312. 1918.
13. LOVE, H. H. A modification of Student's table for use in interpreting experimental results. Jour. Amer. Soc. Agron. **16**: 68–73. 1924.
14. ————, and A. M. BRUNSON. Student's method for interpreting paired experiments. Jour. Amer. Soc. Agron. **16**: 60–68. 1924.
15. RANDS, R. D. Early blight of potato and related plants. Wis. Agr. Exp. Sta. Res. Bul. 42. 1917.
16. ————. The production of spores by *Alternaria solani* in pure culture. Phytopath. **7**: 316–317. 1917.
17. RIDGWAY, ROBERT. Color standards and color nomenclature. 43 pp., 53 pl. Washington, D. C., 1912.

RELATION OF SPECIES OF PYTHIUM TO THE ROOT-ROT DISEASE OF SUGAR CANE

C. W. Edgerton, E. C. Tims and P. J. Mills

The trouble of sugar cane known variously as root rot, root disease, root-rot complex, and growth-failure complex has been known and studied in sugar-cane countries for a long period. The most prominent symptoms include the failure of the plants to grow satisfactorily, the light yellow color of the leaves, and the decay of the root system. The poor growth, always characteristic of root rot, results from the failure of the root system to function satisfactorily. In severe cases, the roots may be so extensively rotted that a whole stool may be pulled from the ground with very little difficulty. In less severe cases, the roots show only rotted ends and few laterals.

Since many factors may influence the development and functioning of the root system, it is not reasonable to presume that the root-failure trouble may always be due to a single cause or be produced by the same factors in every country. As a matter of fact, various workers at one time or another have suggested many causes for the root rot. Among others, these include the chemical and physical condition of the soil, the presence of toxins in the soil, and the attack by certain soil-inhabiting animals and fungi. While the present article treats only of the relation of certain fungi to the root rot as it occurs in Louisiana, it must be remembered that there are also a number of other important contributing factors.

A study of the literature relating to the root rot of sugar cane shows that a number of different fungi have been associated with the disease. Of these, those given most attention at one time or another include species of Marasmius, Rhizoctonia, and Pythium. While none of these species have been entirely eliminated from the problem, most of the work now being carried on in different sugar countries is with species of Pythium. Inoculation experiments with most of the other fungi have not given consistent and satisfactory results.

Fungi of the Pythium type have been reported at various times on cane roots, but the first satisfactory evidence that any of these had anything to do with the root disease was presented by Carpenter (3) in 1919. He found coenocytic mycelium and oospores in roots of cane suffering from the Lahaina or root rot and associated this mycelium with the flabby, rotten root tips characteristic of root rot. He also isolated the fungus and was able to obtain typical rotting of roots by pure-culture inoculations. (4, 5).

In 1924, Bourne (1) reported a fungus on cane roots in Porto Rico, which he considered very similar to the Pythium described by Carpenter

in Hawaii. Bourne, however, reported the occurrence of numerous oospores in his cultures, this being different from the observations of some of the other investigators.

The relation of Pythium fungi to the root disease, as it occurs in Louisiana, has been treated in articles by Edgerton and his coworkers (8, 9, 10) and by Tims and Mills (13). In these articles, it was stated that numerous fungi were found associated with the decay of cane roots but that inoculation tests were unsuccessful with all except certain species of Rhizoctonia and some Pythium forms. As the Rhizoctonia forms were thought to be of only minor importance, most attention was paid to the Pythiums which were found associated with decaying cane roots.

In the present paper are given the results obtained at the Louisiana Agricultural Experiment Station from more than three years' work on one phase of the root rot, *i.e.*, the relation of certain species of Pythium.

RELATION OF PYTHIUM TO ROOT-ROT DISEASE

The roots of cane plants showing symptoms of root rot are always affected to a greater or less degree. Many of the main roots, both primary and secondary, are short and stubby, due to a decay of the tip portions. As the root tips decay, branches attempt to form back of the diseased portions, but these also usually become infected. Consequently, instead of the long straight roots, characteristic of healthy plants, roots of affected plants are short, crooked, and branched. Furthermore, the small fibrous roots, usually abundant on healthy plants, are few on diseased plants. They seem to rot off as rapidly as they form. In all cases, the young, most rapidly developing portions of the roots are the parts most subject to decay. The affected root tips are grey to brownish in color and are soft and flabby. In the diseased roots, coenocytic mycelium, and oospores characteristic of the genus Pythium, commonly occur.

A root-rot condition similar to that occurring in the field has been produced many times by inoculating young plants in sterilized soil with pure cultures of a Pythium, which occurs commonly on decaying roots in the field. For these tests, soil in six-inch pots was sterilized in an autoclave. After the soil had cooled, about one to two inches was removed from the top and the inoculum which consisted of an agar plate culture of the Pythium was mixed with the soil remaining in the pot. Then seed pieces containing good buds were placed in the pots and covered with the soil which had been removed. As the roots developed, they immediately came in contact with the Pythium-infested soil.

Hundreds of inoculations have been made by this method and in all cases the results have been consistent. As soon as the roots began to form, they became infected with the Pythium and showed the typical soft, flabby

rot characteristic of the disease in the field. Usually, very few roots developed and consequently the young plants growing in the inoculated soil made a very poor growth. In figure 1, the plants from a single inoculation

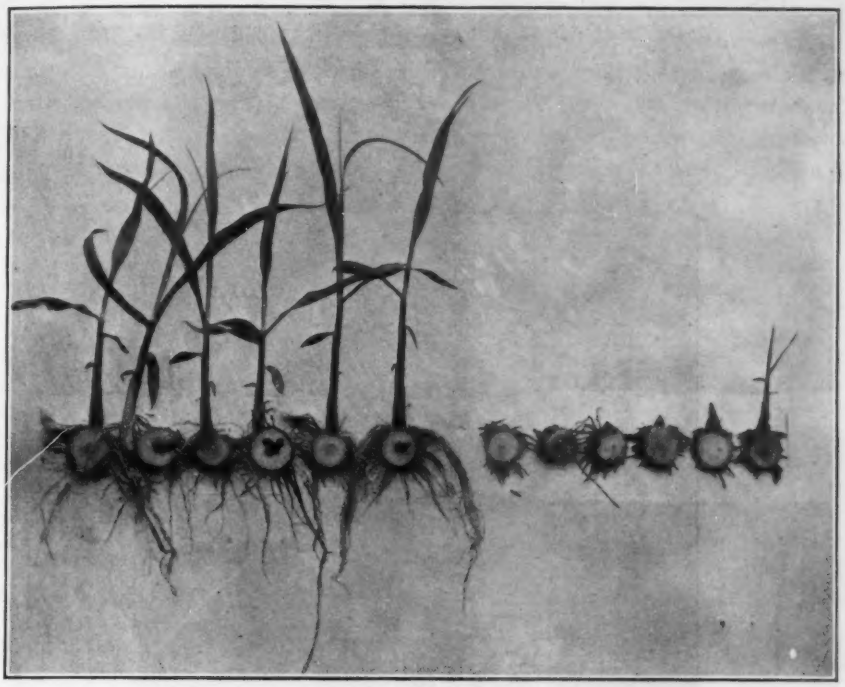


FIG. 1.—Effect of Pythium on young cane plants in sterilized soil. Plants on right, inoculated; those on left, not inoculated. Growth from February 9 to March 9, 1927.

test are shown. The plants on the left were not inoculated while those on the right were inoculated with a Pythium culture.

Inoculation tests have been made also during every month in the year. In many of the sugar-producing countries, it is recognized that the rotting of cane roots is more severe during the cooler, wetter seasons. In Louisiana, root rot is always more serious in seasons following winters of heavy rainfall. In the inoculation tests, the roots of cane have been rotted by the Pythium in both winter and summer, but during the warmer weather, the plants have been able to make a fairly satisfactory growth in spite of it.

Inoculation tests have also been made on all of the common cane varieties, including the resistant P.O.J. canes. The roots of all of the varieties, even of those which show a marked resistance to the disease in the field, have been readily attacked by the Pythium.

These inoculation tests have shown that under certain conditions, some Pythium species are capable of rotting the roots of cane and of producing a rot similar to that commonly seen in the field.

PLANTS AFFECTED BY PYTHIUM

As reported in previous papers (10, 13), the species of Pythium which attack cane also attack certain other members of the grass family but, as far as is known at present, do not attack plants outside this family. Corn, sorghum, and wheat are very susceptible and oats (Texas Red Rust Proof) to a slightly less degree. (Figs. 2 and 3). In the numerous inoculation ex-

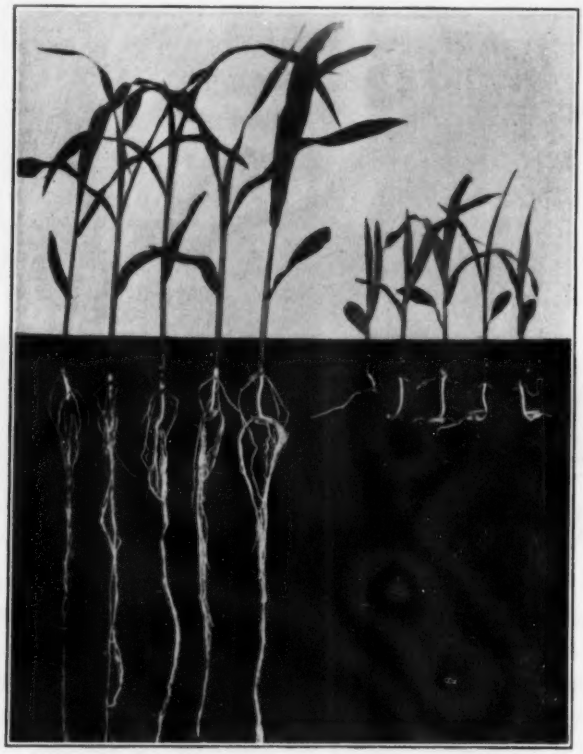


FIG. 2.—Effect of sugar-cane Pythium on young corn plants in sterilized soil. Plants on right inoculated at planting time; those on left were not inoculated. Growth from June 6 to June 17, 1927.

periments carried on in sterile soil, the roots of these plants have been affected in a manner very similar to those of cane and the plants have made a very poor growth. Such legumes as soybeans and cowpeas do not seem to be injured by the cane Pythium. It is interesting to note, however, that

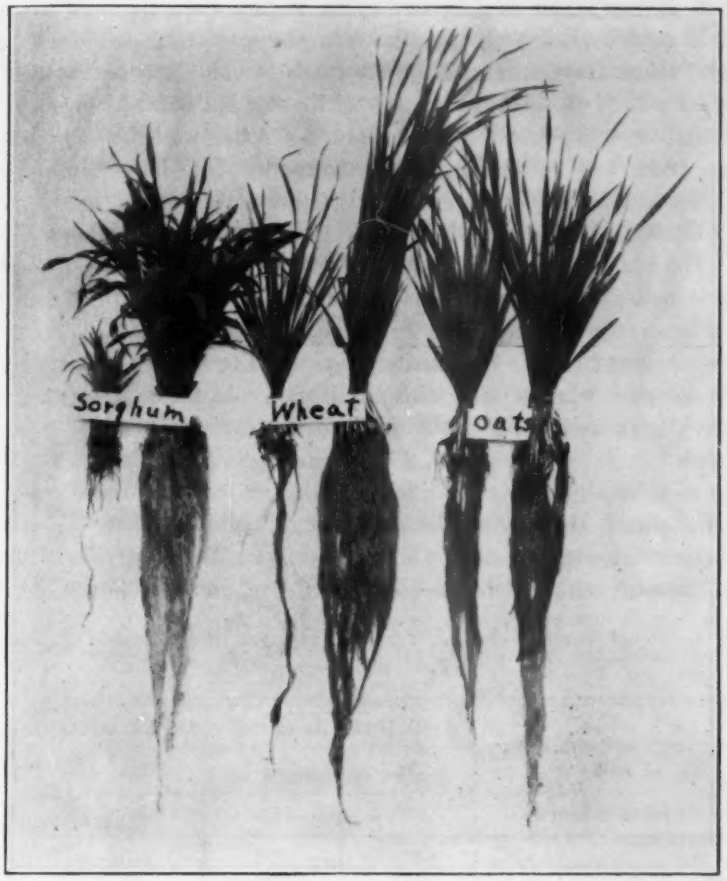


FIG. 3.—Effect of sugar-cane Pythium on sorghum, wheat, and oats in sterilized soil. In each group, the plants on the left were inoculated at planting time; those on the right were not inoculated. Growth from April 19 to May 4, 1928.

there are Pythium parasites of legumes in Louisiana but these do not attack members of the grass family.

The relation of Pythium to the rotting of corn roots has also been mentioned in recent years by Valleau (14), Branstetter (2), Johann (12), and Drechsler (7). These investigators noticed Pythium on the roots of corn and some of them isolated the fungus. Branstetter, in 1927, stated that the fungus found in Missouri was very similar to the Pythium reported by Carpenter in Hawaii.

FUNGI ON CANE ROOTS

In order to determine what fungi are present on cane roots and also the relative abundance of each, a number of surveys have been made over the

cane belt of Louisiana at different times during the past four years. In each field examined, a composite root sample, consisting of more than fifty roots and taken from several different stools of cane, was obtained. These roots were placed in soil-sample cans with sufficient soil to keep them from drying and were brought to the laboratory. After washing in water, small sections, from 5 to 10 mm. in length, were cut from 50 different diseased roots. These were then dipped in a 1–1000 corrosive-sublimate solution, washed in sterile water, and transferred to petri dishes containing bean-pod agar. The plates were incubated at 25° C. to 30° C. for two to eight days and examined. The roots were usually cultured within 48 to 72 hours after being taken from the field.

In some of the earlier reported surveys (9, 13) some of the agar used in the culture work was acidified with lactic acid. As the acid had a tendency to retard the growth of certain of the Pythium forms, it was not used in the later work.

The relative abundance of different fungi on cane roots in Louisiana is shown in table 1, this giving the results obtained from three different surveys during the growing season of 1927. From these surveys, 7890 roots were cultured from 263 fields located in different parts of the cane belt.

TABLE 1.—*Fungi found on cane roots from surveys over the sugar belt of Louisiana in April, June, and August, 1927*

	April, 1927	June, 1927	August, 1927	Total
No. of fields	52	51	160	263
No. of roots cultured	1560	1530	4800	7890
Pythium sp.	273	292	915	1480
Rhizoctonia sp.	217	130	468	815
Marasmius plicatus	16	1	18	35
Trichoderma sp.	300	270	670	1240
Cunninghamella sp.	31	65	196	292
Cephalosporium sp.	45	38	102	185
Aspergillus sp.	36	45	196	277
Fusarium sp.	78	96	171	345
Penicillium sp.	36	28	215	279
Rhizopus sp.	69	69	221	359
Acrothecium sp.	22	53	164	239
Alternaria sp.	26	33	120	179
Phoma sp.	18	16	114	148
Mucor sp.	106	96	272	474
Sclerotium (pink)	7	14	58	79
Sclerotium (black)	7	26	86	119
Oospora sp.	8	15	73	96
Other fungi	107	230	452	789
Free of fungi	367	160	488	1015

An examination of the table shows that a number of different fungi occur quite abundantly on cane roots. Many of these are known to be only saprophytic and have little, if any, bearing on the root-rot problem. Of those known to be parasitic to a greater or less degree, the forms of Pythium were most abundant. Of the 7890 roots cultured, forms of Pythium were observed on 1480. Undoubtedly, many more of the roots were infected with Pythium but growth in the petri dishes was obscured by fast-growing saprophytes. The table shows that forms of Pythium are widely distributed and occur in abundance in the Louisiana cane soils.

FREQUENCY OF PYTHIUMS ON CANE VARIETIES

Certain varieties of cane are known to be very tolerant to the root rot. In Louisiana, varieties like P.O.J. 213 and P.O.J. 36 will make a fairly satisfactory growth under root-rot conditions that will practically stop growth in varieties like Purple and D 74. On this account, it is important to know the occurrence of Pythium on the roots of the different varieties.

In the surveys made in 1927, root samples were taken from a number of the more common and interesting varieties in the different sections of the State. The occurrence of Pythium on the roots of the different varieties is given in table 2. The table shows the percentage of the roots on which Pythium was observed. In each case, fifty roots were cultured from each field.

TABLE 2.—*Percentage of roots of different cane varieties from which species of Pythium were obtained in culture. 1927*

Variety	April, 1927		June, 1927		August, 1927	
	No. of fields	Pythium percentage	No. of fields	Pythium percentage	No. of fields	Pythium percentage
Purple	6	6.1	11	21.6	41	19.8
Striped	6	16.1	4	22.5	4	11.6
D 74	7	8.5	13	17.6	29	21.6
D 95					2	16.6
P.O.J. 36	1	40.0	2	26.6	8	15.0
P.O.J. 213	3	23.3	2	30.0	14	24.5
P.O.J. 228	1	16.6				
P.O.J. 234	18	22.4	14	15.0	27	18.5
P.O.J. 979	2	36.6	2	28.3	8	16.6
L–511	1	30.0			1	10.0
Uba	2	16.6			2	10.0
Cayana 10			3	11.1	2	1.6
Crystallina	1	33.3				

An examination of the table shows that the roots of all of the varieties carried a high percentage of Pythium infection. While there are some variations in the table, these probably are due to the relative amount of Pythium infestation in the different fields rather than the susceptibility of the different varieties. It would seem that Pythiums are as frequent and as abundant on the varieties tolerant to root rot as on the susceptible varieties.

PARASITISM OF DIFFERENT PYTHIUMS

In the several surveys made previous to 1928, no attempt had been made to separate the different types of Pythium found on cane roots. Differences between various cultures had been observed, and also by inoculation experiments it had been determined that certain of the cultures, although isolated from cane, were not parasitic on cane roots.

To determine the distribution and relative abundance of the parasitic forms, a survey was made during January and February, 1928. A composite sample of 50 roots was collected from each field and cultured as in the previous surveys. Pure cultures were obtained of as many of the Pythiums as possible. While some of the Pythiums were lost through contamination of the original plates with fast-growing saprophytes, enough were isolated to give the desired information.

To determine the pathogenicity of the cultures, short-cut methods were devised. To test cultures on cane roots in pots in the greenhouse takes about thirty or more days for satisfactory results. From previous investigations involving many cultures, it had been found that those cultures parasitic on cane are also parasitic on corn. As an inoculation test can be run with corn in about ten days, it seemed just as satisfactory to use this plant to test the parasitism of the large number of Pythium cultures. The effect of different cultures of Phythium on corn is shown in figure 4.

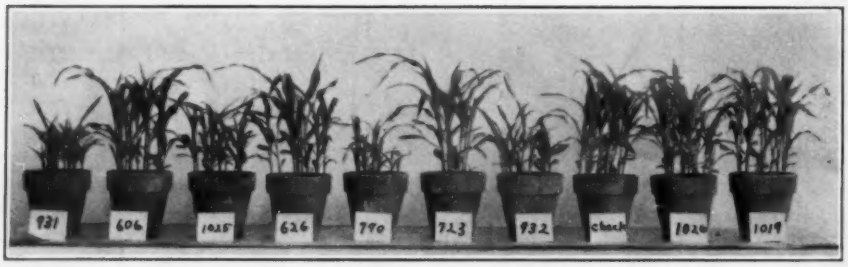


FIG. 4.—Effect of different cultures of Pythium on corn. Cultures 931, 1025, 932, 1026, isolated from cane rots; 606 from spinach; 626 from rhubarb; 790 from corn (received from Miss Johann); 723 from orange; 1019 from cotton.

A still shorter method also was devised which at the present time is being used almost exclusively for parasitism tests and may be found useful to others working with Pythiums. Corn kernels were sterilized in a mixture of corrosive sublimate (1–1000) in 50 per cent alcohol, according to the method used by Chen (6), and then placed in sterile petri dishes containing moistened filter paper. After the hypocotyls had reached a length of two to three centimeters, they were ready for inoculation. Figure 5, A, shows a petri dish containing the germinating kernels as they were used

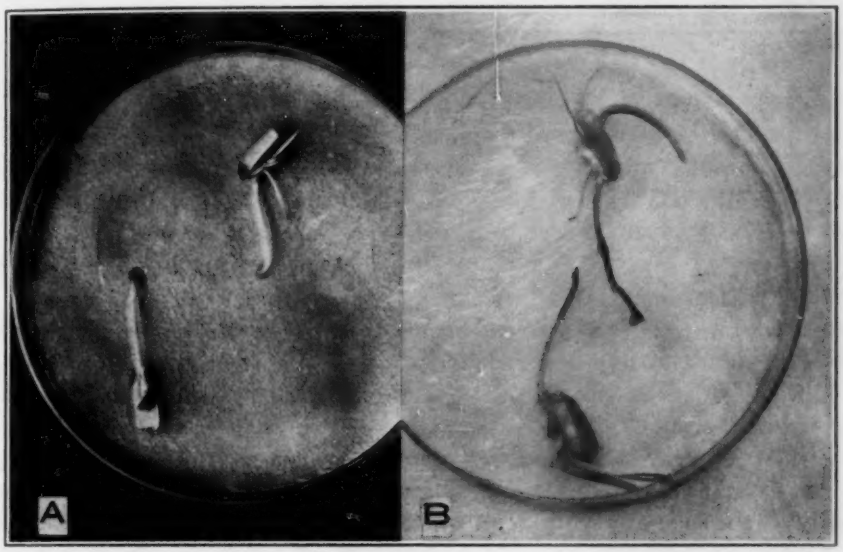


FIG. 5.—Rot produced by a parasitic Pythium on corn roots in petri dishes.
A. Condition of roots at time of inoculation.
B. Condition 48 hours after inoculation.

for inoculation. Sections of agar, 5 to 10 mm. square, from a young, vigorously growing plate culture were placed upside-down on the root tips. The roots were incubated at 25° C. for 48 hours and examined.

In the petri-dish tests, some cultures caused within 24 to 48 hours a complete collapse and decay of the part of the root in contact with the agar. (Fig. 5, B.) These were the same cultures that severely decayed the roots of corn and cane in pot experiments. A few cultures produced only a slight decay of the cortical cells, while others did not seem to affect the roots at all.

In testing the parasitism of the cultures obtained from the survey, both the pot method with corn and the petri-dish method were used. The results from the survey and from these tests are given in table 3.

TABLE 3.—*Occurrence and parasitism of species of Pythium from survey made in January and February, 1928*

Variety	Parish	Occurrence		Parasitism, pot method		Parasitism, petri-dish method	
		Number roots cultured	Number with Pythium	Number cultures tested	Number parasitic	Number cultures tested	Number parasitic
P.O.J. 234	St. John	50	28	25	5	24	6
P.O.J. 36	St. John	50	22	19	17	19	16
P.O.J. 213	St. John	100	36	29	13	28	9
P.O.J. 213	Lafourche	100	32	29	29	27	27
P.O.J. 36	Lafourche	50	38	37	23	36	23
P.O.J. 213	Assumption	100	50	46	34	45	36
P.O.J. 228	Assumption	50	32	21	20	21	20
D 74	Iberville	50	30	20	17	19	17
P.O.J. 234	Iberville	50	12	12	11	11	10
Purple	Iberville	50	32	27	20	26	19
P.O.J. 36	Iberville	50	32	23	23	23	23
P.O.J. 213	Iberville	50	26	22	13	23	13
P.O.J. 234	Iberville	150	103	70	64	70	64
P.O.J. 213	Iberville	50	43	37	35	37	33
P.O.J. 826	W. Baton Rouge	50	23	9	9	9	9
P.O.J. 213	W. Baton Rouge	100	30	22	21	22	21
P.O.J. 36	W. Baton Rouge	50	16	11	8	11	8
P.O.J. 36	Terrebonne	100	38	32	31	32	31
P.O.J. 213	Terrebonne	50	32	27	21	24	19
P.O.J. 213	St. Mary	50	20	20	14	19	13
P.O.J. 213	St. Mary	50	32	19	18	19	18
Purple	St. Mary	50	28	14	14	14	13
P.O.J. 234	St. Mary	50	28	22	22	22	20
P.O.J. 234	St. Martin	100	68	37	33	36	31
P.O.J. 213	St. Martin	50	20	12	10	12	9
P.O.J. 36	St. Martin	50	22	20	17	17	16
P.O.J. 234	St. Martin	50	28	23	21	23	21
P.O.J. 213	Lafayette	50	24	22	20	22	20
P.O.J. 234	Lafayette	50	18	7	5	7	2
P.O.J. 36	Lafayette	50	24	19	17	18	12
Total		1900	967	723	605 (82%)	716	579 (80%)

In the survey, which covered the main portion of the cane belt, 1900 roots were collected and cultured. The percentage observed infected with Pythium varied from 24 to 86. In the inoculation experiments, 733 cultures were tested by the pot method and 715 by the petri-dish method. The results obtained by the two methods agree very closely. By the pot method 82 per cent were found parasitic and 80 per cent by the petri-dish method.

The results obtained from this survey and the inoculation tests show that forms of Pythium capable of attacking the roots of cane and corn are widespread over the cane belt of Louisiana.

TYPES OF PYTHIUM OBSERVED

Among the large number of Pythium and Pythium-like cultures isolated from sugar-cane roots, there have been found a number of different types. Some of these have been studied to a certain extent but no attempt has as yet been made to identify them. While it is very probable that some of them are undescribed species, there has been no opportunity to compare them with accurately determined cultures.

The cultures isolated from cane roots differ not only in their ability to attack cane roots, but also in their morphological characters. As has been noted on a previous page, from a parasitism standpoint, some cultures are extremely virulent on roots of cane and corn, others are mildly parasitic, while still others have practically no effect on living root tissues of these plants. From a morphological standpoint, the cultures differ in regard to the abundance of oospores and conidia formed in culture media, the size of these fruiting bodies, the markings on the surface of the oospores, and the characters of the mycelial threads.

Of the many Pythium types which have been isolated possibly four are worthy of mention, either because of their frequency on cane roots or their parasitic characteristics. It is possible that some of these may represent groups of species rather than single ones. These are briefly described.

1. Of the Pythiums isolated from cane roots, but later found to be nonparasitic to cane and corn, one in particular was common. This form produces smooth oospores, 23–30 μ in diameter, in great abundance on almost any of the common culture media. While not parasitic to cane or corn, some of the cultures, at least, were parasitic on soybeans and cowpea roots. While these nonparasitic forms can have no relation to the root disease, it is possible for the oospores to develop and later be found in dead cane roots. Consequently, the presence of oospores in corn and cane roots does not necessarily mean that parasitic forms of Pythium are present.

2. Another type of Pythium, characterized by abundant production of smooth oospores, but differing from the above form in being moderately

parasitic on cane roots, has been obtained occasionally in pure culture. This Pythium produces oospores readily in pure culture. Several antheridia attached to a single oospore have been frequently noticed in cultures of Pythium of this type. How important this fungus is in the root-rot problem has not been determined.

3. A type of Pythium characterized by spiny oospores has also been observed occasionally. The oospores occur abundantly in the tissues of the roots, including the central stele. A similar fungus has recently been reported by Carpenter in Hawaii. It is not found sufficiently abundant in Louisiana to be important.

4. The Pythium form isolated in Louisiana, which is the most actively parasitic to cane and corn roots and apparently the most important in the root-rot problem, does not produce fruiting structures of any kind readily on ordinary culture media. Oospores have been observed in roots from the inoculation experiments, in roots from corn plants growing under aseptic conditions in large test tubes and also on fresh clean cane roots placed in sterile water in petri dishes and inoculated with a pure culture. In a few cases in the roots, the oospores have been quite abundant. The mycelium of this Pythium also is quite characteristic. Beside the ordinary type of mycelium, the fungus produces numerous globular, lobed or irregular bodies which may be considerably branched. (Plate XI, E and F.) As these large bodies develop, often the protoplasm from the hypha on which they form flows into them and the hypha is left as an empty tube. Each one of these masses is then separated from the old colony. These large bodies, which are doubtless the same as the enlarged hyphae often seen in cane root tissues (Plate XI, D), have been described by other investigators, including Carpenter and Branstetter. These bodies resemble the prosporangia described by Edson (11), but all attempts to produce zoospores from them have been unsuccessful. As far as has been observed, they germinate by means of small germ tubes. The identity of this Pythium is at present uncertain. In Hawaii, Carpenter is calling the fungus *Pythium aphanidermatum*. As brought out in a previous paper (10), this determination cannot be correct because *P. aphanidermatum* attacks a number of plants on which the sugar-cane Pythium is nonparasitic. Drechsler (7), in a recent paper, also states that the corn and cane Pythiums are distinct from *P. aphanidermatum*.

A culture of a corn Pythium received from Miss Johann of the United States Department of Agriculture and a culture of a Hawaiian-cane Pythium have also been compared with the Louisiana cultures. While similar in many ways, these cultures have shown some differences. Conidia are produced in pure cultures much more readily than in cultures of the

parasitic Pythium from Louisiana. It is not yet possible to state whether or not the cultures are distinct enough to represent separate species.

LOSS OF VIRULENCE

In the course of the work with Pythiums, it has frequently been observed that all cultures of parasitic Pythiums are not equally parasitic. Certain cultures would practically prevent the development of roots of cane or corn in sterilized soil, while others would develop slower, often permitting the growth of a fairly good root system. This condition might mean the presence of different species or different strains or it might mean that the fungus could lose its virulence when grown under certain conditions.

The inoculation experiments carried on during the period of more than two years have possibly helped to explain this condition. One of the early cultures isolated in the summer of 1926 was extremely virulent at the time of isolation. This has been kept in culture on bean-pod agar for more than two years. As it has been used in inoculation experiments almost constantly, it has been possible to observe any change in virulence. For a period of about a year, no change in the virulence was noted. During the second year, however, the virulence gradually declined. After two years, the culture was only moderately parasitic. At that time, even under conditions very favorable for the fungus, corn or cane roots would make a fair growth in inoculated soil. The roots would become somewhat discolored and a few would show the soft flabby tips but the effect was far less marked than with the same culture on an earlier date. Freshly isolated cultures used at the same time, as a check, were as parasitic as this culture was known to be at first. As this culture originally came from a single mycelial thread transferred from a plate, this condition cannot be explained in any other way than that the culture gradually lost its virulence. It is possible that the presence of weakly parasitic forms in the field might be explained in the same way.

DISCUSSION

Although it has been demonstrated that certain species of Pythium are able to cause a severe rotting of cane roots under controlled conditions in sterilized soil, it is not easy to determine the exact relation of these fungi to the root rot as it occurs in the field. In cane fields severely injured by root rot, large numbers of roots may be found showing pale, greyish or brownish, flabby ends. These roots contain mycelium and spores of Pythium and are very similar to roots previously inoculated with pure cultures in sterilized soil.

On the other hand, the surveys made in Louisiana show that these same species of Pythium are widely distributed over the sugar-cane belt occurring

as abundantly, apparently, in fields of thrifty and seemingly healthy cane, as in fields of chlorotic, stunted cane.

Furthermore, it is known that certain varieties of cane are much more tolerant to root rot than others and will make a fair growth in fields in which susceptible varieties will be very badly affected. In Louisiana, varieties like P.O.J. 36 are tolerant, while varieties like Louisiana Purple and D 74 are susceptible. In Hawaii, a similar condition exists and the variety H 109 has practically replaced the Lahaina cane in districts where the Lahaina disease is severe. Yet, in Louisiana, it has been found that roots of varieties like P.O.J. 36 and P.O.J. 213 are readily attacked by species of Pythium in inoculated sterilized soil.

The distribution of root rot in differently located and treated fields and its occurrence from year to year are also of considerable importance. It may be severe in one field and of minor importance in another nearby, and its occurrence in a field one year does not necessarily mean that it will be troublesome with the next crop. Usually, in infected fields, there are areas of good and poor cane. The disease is also very much more trouble some years than others. In some seasons, a very large percentage of the crop shows the root-rot symptoms, while in other years the disease shows only in favored spots.

If species of Pythium are to be recognized as organisms responsible for a considerable portion of the decay of the cane roots, it is necessary to explain why they are sometimes active and other times not. It must necessarily follow that there are other factors present which may make the plant itself more resistant or else make the fungi less effective. Species of Pythium are to a degree facultative parasites and it is reasonable to suppose that their activity as parasites depends to a considerable extent on temperature and moisture conditions, on physical and chemical conditions in the soil, on the reactions of various soil organisms, and on the vitality of the cane itself.

Certain of the factors which influence the development of root rot are fairly well known. For instance, it is known that the disease is always severe following winters of heavy rainfall (9) and that it is favored by poor drainage conditions.

At present, an intensive study is being made of the factors influencing root rot and the parasitism of species of Pythium. These include among others, temperature and moisture conditions and the effect of the application of both organic and inorganic substances to the soil. From these studies it is expected that a better knowledge of the conditions favorable or unfavorable to root-rot development will be determined.

The evidence at present, mainly accumulated from field observations extending over a period of several years and supplemented by results ob-

tained from numerous inoculation experiments, seems to indicate that certain species of Pythium do cause a serious rotting of cane roots and, to a certain extent, cause a killing of the buds, while the cane stalks are covered with soil during the winter and early spring. However, the severity of the attack seems to depend very largely upon the vigor of the cane plants and upon certain environmental conditions such as temperature, soil moisture, and the physical, chemical, and biological conditions of the soil.

SUMMARY

1. A disease of sugar cane known as root rot or root disease occurs in many of the sugar-producing countries of the world. The roots of the cane decay and cause the plants to make a poor and unsatisfactory growth.

2. The ends of the roots and the young laterals are the most subject to decay. Usually the tips of the roots become soft and flabby due to the decay of the stele as well as the cortex.

3. In Louisiana, species of Pythium are usually present in the decaying cane roots.

4. A decay of cane roots in sterilized soil, similar to that which occurs in the field, has been produced consistently in numerous inoculation experiments with pure cultures of Pythium. In the inoculation tests, roots of resistant varieties rotted as readily as did those of susceptible varieties.

5. Certain other members of the grass family, including corn, sorghum, wheat, and oats are attacked by Pythium as readily as is sugar cane. Legumes are not attacked by the same species.

6. Pythium species are readily isolated from cane roots. From the results of thousands of root cultures, it has been determined that these fungi occur in great abundance, being the most common organisms present.

7. All cultures of Pythium obtained from cane roots are not parasitic to cane. In tests about 80 to 85 per cent. have been found parasitic.

8. Parasitic Pythiums occur on roots of both thrifty and stunted cane.

9. Several types of Pythium have been found on cane roots. One of these seems to be of the most importance. Its identity has not been determined.

10. The ability of Pythium to attack cane roots seems to depend to a considerable extent on environmental and other factors.

LOUISIANA STATE UNIVERSITY,
BATON ROUGE, LA.

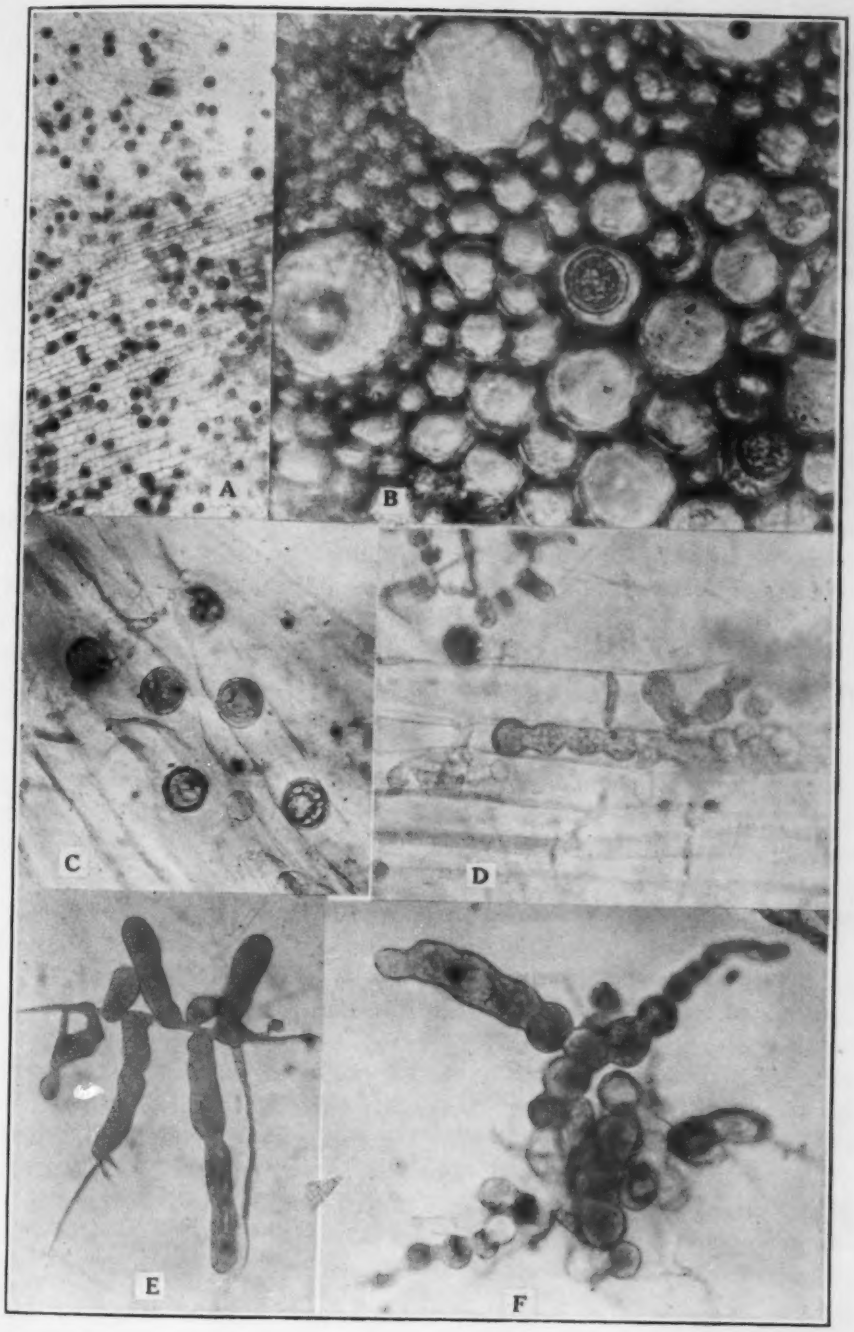
LITERATURE CITED

1. BOURNE, B. A. Morphological similarity between the Pythium-like fungus found associated with diseased sugar-cane roots in Hawaii and Porto Rico. Jour. Dept. Agr. Porto Rico **8** (2): 61–70. 1924.
2. BRANSTETTER, B. B. Corn root rot studies. Missouri Agr. Exp. Sta. Res. Bul. 113. 1927.
3. CARPENTER, C. W. Preliminary report on root rot in Hawaii. Hawaii Agr. Exp. Sta. Press Bul. 54. 1919.
4. ————————. Pythium in relation to Lahaina disease and pineapple wilt. Hawaiian Planters' Rec. **23**: 142–174. 1920.
5. ————————. Morphological studies of the Pythium-like fungi associated with root rot in Hawaii. Bul. Exp. Sta. Hawaiian Sugar Planters' Assoc. Bot. Ser. **3**: 59–65. 1921.
6. CHEN, C. C. Internal fungous parasites of agricultural seeds. Md. Agr. Exp. Sta. Bul. 240: 81–100. 1920.
7. DRECHSLER, CHARLES. **Pythium arrhenomanes**, n. sp., a parasite causing maize root rot. Phytopath. **18**: 873–875. 1928.
8. EDGERTON, C. W., W. G. TAGGART, and E. C. TIMS. The sugar cane disease situation in 1923 and 1924. La. Agr. Exp. Sta. Bul. 191. 1924.
9. ————————, and E. C. TIMS. Investigations on the sugar cane disease situation in 1925 and 1926. La. Agr. Exp. Sta. Bul. 197. 1927.
10. ————————. The fungus, Pythium, in relation to the root rot of sugar cane. Reference Book Sugar Industry of the World **6**: 39–40. 1928.
11. EDSON, H. A. *Rheosporangium aphanidermatus*, a new genus and species of fungus parasitic on sugar beets and radishes. Jour. Agr. Res. **4**: 279–292. 1915.
12. JOHANN, HELEN, J. R. HOLBERT, and J. G. DICKSON. A Pythium seedling blight and root rot of dent corn (Abst.). Phytopath. **16**: 85. 1926.
13. TIMS, E. C., and P. J. MILLS. Some fungi associated with the root disease of sugar cane in Louisiana. Reference Book Sugar Industry of the World. **5**: 33–37. 1927.
14. VALLEAU, W. D., P. W. KARRAKER, and E. M. JOHNSON. Corn root rot—a soil-borne disease. Jour. Agr. Res. **33**: 453–476. 1926.

PLATE XI.

Mycelial and fruiting characters of species of Pythium from sugar cane.

A. Numerous oospores in cane roots. 64X.
B. Oospores in cane roots, natural infection. 353X.
C. Oospores in cane roots from inoculation test. 300X.
D. Swollen cells of Pythium in cane roots from inoculation test. 300X.
E. Swollen cells from Pythium culture isolated in Louisiana. 300X.
F. Swollen cells from Pythium culture from Hawaii. 300X.


A
B
C
D
E
F

PLOT ARRANGEMENT TO DISTRIBUTE NEIGHBOR INFLUENCE IN FIELD TRIALS

CARL HARTLEY

INTRODUCTION

Competition between adjacent plots has frequently been emphasized by agronomists as a disturbing factor in field trials. An excellent brief statement of the difficulties involved is that of Stadler (13, p. 6–8). Competition is by no means the only kind of neighbor influence that has been recognized. Particularly, in pathological and entomological experiments, the results on a plot may be materially influenced by the parasite harbored in a neighboring plot or by the experimental treatment that has been given a neighbor. An especially good example of such interference is found in a series of experiments on rahar (*Cajanus indicus*) described by McRea (8, p. 45, 46). It was found that the wilt caused by *Fusarium udum* spread through the soil 9 feet in a single season. Certain plots were so affected by the wilt's spreading into them from their neighbors that the experiment threw no light on the fertilizer problems for which it had been conducted. In the same way, when parasites of aerial plant parts are present, inclusion of a highly susceptible variety in a variety trial may subject the varieties planted adjacent to it to abnormally heavy infection. This effect may, of course, be offset in part by decreased competition from the diseased variety. A fertilizer, an irrigation, or a soil-disinfectant treatment may have a direct effect on the margins of neighboring plots; the drift of fungicides or insecticides through the air in spraying and dusting experiments may exert a still more distant neighbor influence. R. D. Rands[1] pointed out that some treatments may act as repellants to harmful insects, snails, or other animals and in this way result in intensified infestation of neighboring plots.

By the usual methods of plot arrangement in experiments containing several replications the varieties or treatments are arrayed in approximately the same order in each series. This means that variety C has varieties B and D for neighbors in several different series or perhaps in every series in the experiment. If variety D is especially inclined to influence its neighbors unfavorably, the yield of variety C will be subject to a systematic error that can not be measured or corrected satisfactorily.

A common method of avoiding or reducing neighbor effects has been to leave planted or unplanted buffer strips between the trial plots, or to discard plot borders in taking results. There are a number of objections to such procedure. It places the plots to be compared farther from one an-

[1] Oral communication.

other than they otherwise would be, with consequent decrease in the value of comparisons. If the buffer strips are left unseeded, they sometimes (3, 4) cause certain of the varieties or treatments under comparison to take a higher or lower rank than that to which they are entitled. The use of buffer strips increases the amount of land and labor required for an experiment. In the Beaven arrangement (1), discarded buffer strips occupy 7/16 of the entire field, while in 3-row plots (13), 2/3 of the field is so sacrificed. Furthermore, particularly in the case of pathological factors, the distance through which influence is exerted may be so great or so uncertain that there will be no assurance of eliminating neighbor influence by any practicable width of buffer.

ARRANGEMENT FOR ROWS OR OBLONG PLOTS

In experiments at the Instituut voor Plantenziekten, Buitenzorg, Java, in 1921, the writer was confronted with the need of testing numerous varieties of peanut for resistance to a soil-borne disease, when the only fields known to be infected heavily and uniformly enough for proper comparisons were too small to permit the use of any kind of buffer strips. Single rows were accordingly taken as experimental plots and all attempt to prevent neighbor influence was abandoned. Instead a special planting arrangement was devised to distribute neighbor influence as equally as possible to all the varieties in the test.

To carry out this arrangement completely, the varieties are arrayed in different order in the different replication series in such a way that if the number of replications equals the number of varieties tested, each variety is in contact twice with each other variety. If, then, variety C should prove dangerous to its associates because it carries heavy disease infection or makes unusually heavy demands on soil moisture, all of the other varieties in the test would be equally subject to the misfortune of being in contact with it. All of them would make a poorer showing than if C were not in the experiment, but there would be relatively little chance that the influence of the C plots would cause any one of the other varieties to take a lower rank than it deserved. The arrangement by which this was accomplished is shown in figures 1 and 2. It has been tested in work with sugar cane by Rands and Stevens (11).

One need for refinement was not at first realized; if variety or treatment C exerts an influence on its neighbors, then not only all the other varieties, but also variety C, itself, must be subjected to that influence if comparisons are to be fair. This can be done only by seeing that each variety comes into contact with itself as often as it does with any other variety.

To understand the system followed in this arrangement, the guard plots, indicated in figure 1 by broken lines, should for the present be ignored, and

	Series 1	Series 2	Series 3	Series 4	Series 5	Series 6	Series 7	Series 8
Guard Plot	A	H	G	F	E	D	C	B
Plot 1	A	H	G	F	E	D	C	B
Plot 2	H	G	F	E	D	C	B	A*
Plot 3	B	A*	H	G	F	E	D	C
Plot 4	G	F	E	D	C	B	A*	H
Plot 5	C	B	A*	H	G	F	E	D
Plot 6	F	E	D	C	B	A*	H	G
Plot 7	D	C	B	A*	H	G	F	E
Plot 8	E	D	C	B	A*	H	G	F
Guard Plot	E	D	C	B	A	H	G	F

FIG. 1. Arrangement of an eight-variety test with each variety twice in intraseries contact with every other variety, and with distribution balanced (center of gravity for each variety coinciding with that for the entire experiment). Plots planted to variety A are starred to make this distribution more evident. The plots inclosed in broken lines are guard plots, which are also useful in subjecting each variety to contact with itself; they are discarded in taking the results of the experiment proper.

	Series 1	Series 2	Series 3	Series 4	Series 5
Guard Plot	A	E	D	C	B
Plot 1	A*	E	D	C	B
Plot 2	E	D	C	B	A*
Plot 3	B	A*	E	D	C
Plot 4	D	C	B	A*	E
Plot 5	C	B	A*	E	D
Guard Plot	C	B	A	E	D

FIG. 2. The arrangement shown in fig. 1, adapted to an odd number of varieties or treatments.

attention paid only to the letters in the solid-line plots. In the first series, variety A is put in the first odd-numbered plot, B in the second odd-numbered plot, and so on. The method expressed generally is to go down through the series filling the odd-numbered plots, and then return, filling the even-numbered plots. The second series is started with variety A in the second odd-numbered plot instead of the first. The third series is started with A in the third odd-numbered plot. In series 2, 3, and 4, one gets back to the top of the series with one or more varieties still unused. He disposes of these by coming down again through the still unfilled odd-numbered plots at the top of the series. The last four series in an 8-variety test are a reversal or mirror image of the first 4; the fifth series is begun by putting variety A in the lowest even-numbered plot, going up while filling the even-numbered plots and then down through the odd-numbered ones. In series 6, A is put in the second even-numbered plot from the bottom.

An odd number of varieties is handled in the same general way. This arrangement for a 5-variety test in shown in figure 2. With an odd number of varieties the plan can not be divided into two mirror-image halves, but the same rule is followed that when A has been used in the bottom odd-numbered plot, it is used in the next following series in the bottom even-numbered plot.

Only intraseries contacts are considered here. Field plots are ordinarily much longer than wide; the interseries contacts are between plot ends and usually can be disregarded, or avoided by buffer strips which will be relatively short, as indicated on the diagrams in figures 1 and 2.

The arrangement as shown by the plots in solid lines in figures 1 and 2 fails to provide self contacts. Self contacts have accordingly been supplied by adding guard plots to each series, shown by the dotted lines. It is usually a desirable practice to put at the ends of an experimental series plots that are sown like the experimental plots but whose yields are not considered when the results are taken. By putting in each of these guard plots the same variety as is used in the adjacent test plot, each variety is subjected to as many contacts with itself as it has with any other variety.

Past attempts to avoid error from neighbor influence had in some cases involved the grouping of closely related varieties, and in other cases the use of different orders in different series (13). The only arrangement encountered in which the order was shifted systematically enough to fully equalize contacts within series was the one devised by Richey for another purpose and shown in part by him (12, table 1, columns 2, 9, 10, 11, and 12). As he pointed out, every variety competed an equal number of times with every other variety; an equal number of self-influenced plots for each variety was also provided by his scheme. His arrangement is not entirely balanced, and to carry out his scheme completely the number of replications must be more than twice the number of varieties tested, requiring $(2M^2 + M)$ plots when M is the number of varieties. He did not favor its further use, but suggested the employment of some method that changed the order in different replications.

The arrangement used by the writer is perfectly balanced when the guard plots are ignored. Maskell (9) has emphasized the value of balanced distribution in field trials. For complete balance, the center of gravity for each variety must coincide with the center of gravity of the experiment as a whole. The A's are starred in figures 1 and 2 to make it easier to judge the distribution of replicates. It will be noted that there is one A plot in every rank and every file; as Fisher has pointed out through Maskell (9, p. 380), in any diagrammatically (2) square arrangement this insures the balance of the distribution.

In adapting the proposed arrangement to fields of different shape, the different series may be shifted in position from that shown in figures 1 and 2 without loss of balance so long as their orientation remains the same. If series are placed end to end, proper placing will provide many of the desired self contacts between varieties without need of guard plots. For example, if series 6 of figure 1 is placed below series 2 but without guard plots, the D plots of each series will be in direct contact with each other and both D plots will then be subjected to self-influence.

The proposed arrangement will be more useful in final tests than in preliminary elimination trials, because of the small number of replications possible in the former. If it is only possible to have half as many replications as there are varieties, in such an experiment as shown in figure 1, only series 1–4 would be employed and contacts would still be completely equalized, though balance would no longer be complete. If it were possible to employ but 3 replications, series 1–3 would be followed, and, while contacts would not be entirely equalized, no pair of varieties or treatments would contact more than once, so that there still would be little error due to neighbor influence.

It is sometimes desirable to have more replications of some things than of others. If, for example, 6 different treatments are to be tested and there are to be 2 untreated plots in each series, the experiment is outlined like an 8-treatment test, and untreated plots are put in place of two of the letters, *e.g.*, D and H, shown in figure 1. At first thought such a procedure seems to provide too many self contacts for the untreated plots, but analysis shows that this is not the case.

Because some effects, such as those resulting from shading or from the drift of fungicides with the wind, are exerted mainly in one direction, it is preferable to have even numbers of objectives in an experiment. In figure 1, showing an 8-variety test, it will be noted that of the 2 contacts between any pair of varieties as A and B, A is above in one case and below in the other. In figure 2, showing a 5-variety test, the result is seen that would always occur in an experiment with an odd number of objectives. In both its contacts with B, A is placed below it.

ARRANGEMENTS FOR TREES OR SQUARE PLOTS

In some experiments, square plots are preferred to oblong plots or rows, *e.g.*, centgener experiments and Beaven's chessboard for small grains. In experiments with woody plants or plants grown in hills a single plant or hill may constitute the experimental unit. In all such cases it is desirable to equalize end as well as side contacts; the contacts that appear in the horizontal axis in the chart, as well as those in the vertical axis.

While no arrangements have been developed that would equalize contacts in the ranks of a chessboard and independently equalize them in the

files, it is not so difficult to equalize the total number of contacts when both the contacts within ranks and those within files are added together. The chessboard shown by Orton and Olson (10) for 5-variety tests, while it scarcely lives up to their claim of eliminating experimental error, is excellent for square plots in reducing the portion of the error that results from neighboring influence. In their arrangement variety 2 has varieties 1 and 3 for its only neighbors in the ranks, and varieties 4 and 5 for its only neighbors in the files, so that when one considers both vertical and horizontal contacts each variety in the test competes with each other variety four times. The arrangement is a good one for square plots. For plots that are much longer than wide, contacts in ranks and contacts in files are not of equal importance and their arrangement is not satisfactory.

Orton and Olson make a sweeping recommendation of magic squares in checkerboard arrangements. These are squares such that, if the varieties are represented by numbers, the sums of the figures in each rank, each file, and each of the two main diagonals will be the same. It is by no means

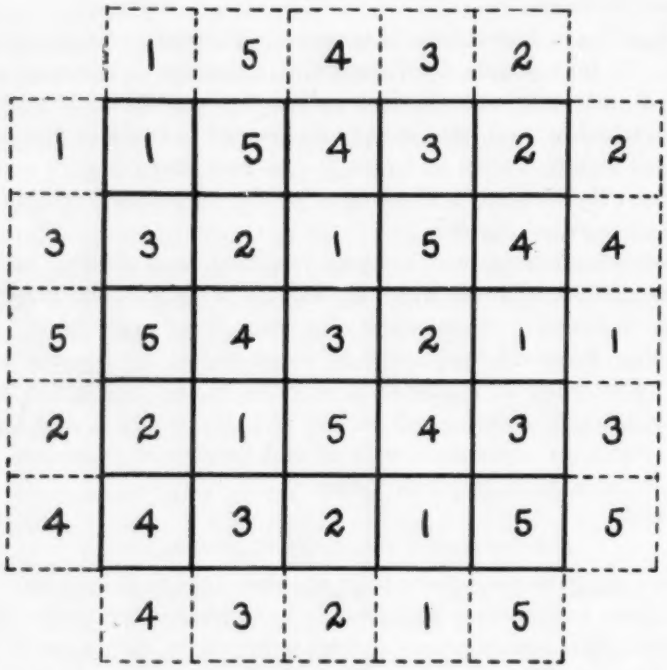


FIG. 3. A five-variety magic square in which each variety comes in contact with each other variety four times, counting both vertical and horizontal contacts. It is taken from Ball (*5*, fig. iv) but has been provided with self contacts by adding guard plots, which are shown by broken lines.

necessary to make a square magic in order to secure good distribution of replicates. Fisher's simpler rule already mentioned (9), that each variety should occur once in each rank and once in each file, is much better. The 6-variety diagram that Orton and Olson show as a magic square, in fact, is not magic because the sums of the diagonals are not equal, but it complies with Fisher's rule and is quite as satisfactory an arrangement as if it were magic. The magic quality gives no assurance of equalization of contacts; nothing could be much worse from the neighbor-influence standpoint than the magic squares obtained by one of De la Hire's methods for first subsidiary squares (5, p. 143), which, if applied to a variety test, would give a variety the same pair of neighbors in both ranks and files throughout an experiment.

A balanced 5-variety square similar to that shown by Orton and Olson is found in Ball's figure iv (5); the arrangement is shown in figure 3, the varieties being designated by numbers instead of letters so that the magic attributes will be apparent. It happens that the writer's 5-variety plan of figure 2 also is magic, while the 8-variety arrangement of figure 1 is not.

Three-variety and 4-variety chessboards with 2-way-contact equalization and balanced distribution are easily devised; samples are shown in figures 4 and 5. Apparently, any 3-variety square that satisfies Fisher's rule (previously mentioned) for balancing the distribution also equalizes neighbor contacts. This is not true for 4-variety squares. The arrangement shown as figure 5 is identical with one shown by Maskell (9, diagram 3), which he dismissed in favor of random arrangements without considering its value in connection with neighbor influence.

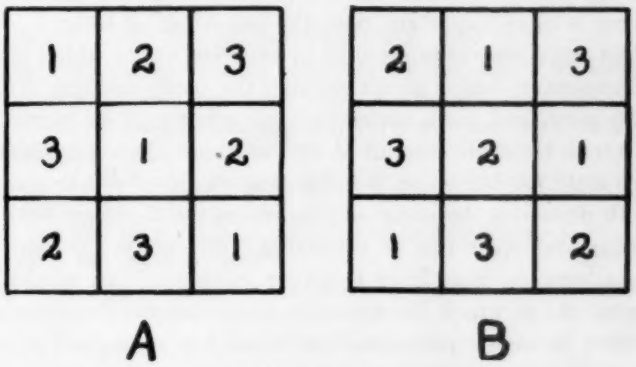


FIG. 4. Three-variety squares, with neighbor contacts equalized when both vertical and horizontal contacts are counted. B is magic, while A is not.

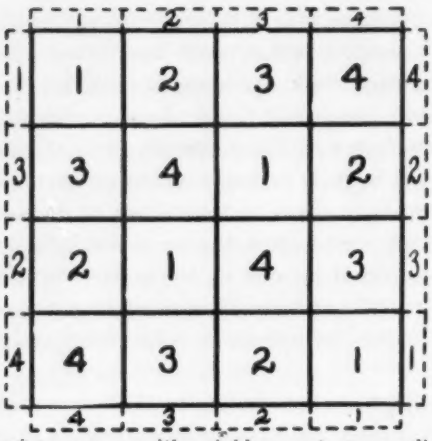


FIG. 5. A four-variety square, with neighbor contacts equalized when both vertical and horizontal contacts are counted. Self contacts are furnished by the guard plots included in the broken lines. The guard plots are made narrow to economize field space.

CONTACT-EQUALIZING VERSUS RANDOMIZED ARRANGEMENTS

Fisher holds randomized plot arrangement superior to any rigid arrangement, because it makes possible a more accurate estimate of the nonsystematic errors. He implies (7, p. 506) that refinement of the estimate of error is more important than the reduction of error.

A contact-equalizing arrangement in experiments in which it makes possible the omission of buffer strips reduces the nonsystematic error both by putting the compared plots closer to each other and by permitting more replications with the same amount of seed and soil. In experiments in which it decreases neighbor influence, it reduces one kind of systematic error, and thereby both decreases the error and increases the accuracy with which the total experimental error can be estimated. Of course the randomized arrangement allows less bias from neighbor influence than would one of the old arrangements in which the varieties of treatments follow each other in the same order in each replication, but would not be so good in this respect as a contact-equalizing arrangement.

The argument of inconvenience raised by Engledow and Yule (6) against randomized arrangement would apply also to a contact-equalizing or any other shifting-order arrangement, but is not believed to be a serious objection. A choice between the two types of arrangement would have to depend somewhat on the amount of neighbor influence expected in the type of experiment to be conducted.

SUMMARY

Competition is by no means the only kind of neighbor influence that may bias results of field trials. This is especially true in experiments on

varietal resistance or prophylactic treatments against diseases or insects.

The use of buffer strips between plots to avoid neighbor influence increases the cost of experimentation; and by decreasing the intimacy of comparisons it reduces their reliability.

Bias due to neighbor effect in replicated trials can be reduced without intraseries buffer strips if each variety or treatment has an equal number of contacts with every other variety or treatment and also with itself. An arrangement is described which attains this end and also satisfies the requirement of balanced distribution.

For small numbers of varieties or treatments it is possible to arrange square plots or single trees or hills in such a way as to equalize or approximately equalize contacts when both interseries and intraseries contacts are counted.

Contact-equalizing arrangement permits a less accurate estimate of nonsystematic error than Fisher's randomizing arrangements; but, under certain circumstances, may so reduce systematic error as to make the computed error more nearly representative of the total experimental error.

FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

LITERATURE CITED

1. Anonymous. On testing varieties of cereals. By Student. Biometrika **15**: 271–293. 1923.
2. ————. Mathematics and agronomy. By Student. Jour. Amer. Soc. Agron. **18**: 703–720. 1926.
3. Arny, A. C. Further experiments in field technic in plot tests. Jour. Agr. Res. **21**: 483–500. 1921.
4. ———— and H. K. Hayes. Experiments in field technic in plot tests. Jour. Agr. Res. **15**: 251–262. 1918.
5. Ball, W. W. R. Mathematical recreations and essays. 506 pp. Edition V. London. 1911.
6. Engledow, F. L., and G. U. Yule. The principles and practice of yield trials. Empire Cotton Growing Review, London **3**: 112–146, 235–267. 1926.
7. Fisher, R. A. The arrangement of field experiments. Jour. Min. Agr. [Gt. Brit.] **33**: 503–513. 1926.
8. McRae, W. Report of the Imperial Mycologist. Agr. Res. Inst., Pusa, Sci. Rpt. (**1923–24**): 41–51. 1924.
9. Maskell, E. J. The technique of plot experiments. Imp. Bot. Conf. London, Rpt. Proc. **1924**: 373–381. 1925.
10. Orton, C. R., and O. Olson. A method of eliminating experimental error in comparative field tests. Penn. Agr. Exp. Sta. Bul. 193: 14–15. 1925.
11. Rands, R. D., and F. D. Stevens. Adequate testing of sugar cane varieties. Sugar Bul. 4: (20): 3–5. 1926.

12. Richey, F. D. Adjusting yields to their regression on a moving average, as a means of correcting for soil heterogeneity. Jour. Agr. Res. 27: 79–90. 1924.
13. Stadler, L. J. Experiments in field plot technic for the preliminary determination of comparative yields in the small grains. Missouri Agr. Exp. Sta. Res. Bul. 49. 1921.

DASYSCYPHA FUSCO-SANGUINEA REHM ON WESTERN WHITE PINE, PINUS MONTICOLA DOUGL.

C. R. STILLINGER[1]

During the last eight years, 1921 to 1928, while scouting for white-pine blister rust, *Cronartium ribicola* Fischer, in the white-pine belt of northern Idaho, northeastern Washington, and northwestern Montana, the writer observed, or had called to his attention, a parasitic disease on western white-pine, *Pinus monticola* Dougl., the gross characteristics of which, on the younger trees, appear in several respects very much like white-pine blister rust. The two are so similar in general appearance that on a superficial examination one may be mistaken for the other. In fact, several areas of western white pine infected with the disease have been erroneously reported as infected with blister rust. Because of the gradual invasion of the white-pine belt by white-pine blister rust and the desirability of locating initial infections of it, any disease which might be confused with it should be reported. Further, since the disease under notice has not within the writer's knowledge been reported as an active parasite on western white pine, it seems worth while to record the following observations on its characteristics, importance, and distribution.

This disease is of still further interest due to its similarity to that caused by the destructive larch canker, *Dasyscypha calycina* (Schum.) Fckl., which has recently been found in the eastern part of the United States (5).

CAUSAL ORGANISM

In nearly all cases where this disease has been observed, a Dasyscypha has been found on the cankers. Saccardo (4) reports over one hundred and fifty species in this genus, but only a small number of them are said to be even mildly parasitic. The only vigorous parasite thus far reported is *D. calycina.*

Underwood (7) reports *Dasyscypha calycina* on *Pinus* sp. Tubeuf (6) says *D. calycina* may parasitize pines and that it occurs in America. Wagner (8) reports *D. calyciformis* (Willd.) Rehm as parasitic on *Pinus sylvestris* L., *Abies pectinata* DC., and *Picea excelsa* Link. Anderson (1) reports *D. resinaria* (Cooke and Phillips) Rehm as a parasite causing cankerous growths on *Abies balsamea* Mill. in Minnesota. According to Jelstrup (3), Jorstad has noted deep cankers on *P. sylvestris, P. montana* Mill. and *P. murrayana* Balf., caused by *D. subtilissima* Sacc. He also found *D. resinaria* on *P. sylvestris.* Of all those which have been reported as

[1] Associate Pathologist, U. S. Dept. Agr.

parasitic, the larch canker, caused by *D. calycina,* is the only one which has been recorded as a vigorous, destructive parasite. A very complete discussion of this parasite is given by Hiley (2).

The Dasyscypha under discussion in this paper is such a vigorous parasite in some areas that it was first thought to be the larch canker established on western white pine in this region. For this reason in all areas where this disease has been observed on white pine, a search has been made for it on larch as well as on other trees. In no case was this fungus definitely found on other species of trees in an area where it occurred on white pine. In northwestern Montana during the summer of 1928, however, a Dasyscypha was found parasitic on a single tree of western larch, *Larix occidentalis* Nutt. A small trunk canker had developed on a young tree about two inches in diameter. No other infections on larch were found. This species appeared to be the same as that on white pine. The measurements of the spores were within the range of those of the white-pine organism, but the color of the apothecia was much lighter.

On a fresh specimen of the white-pine Dasyscypha the stipe and under side of the cup are light brown, while the inside of the cup is a deep orange. On dry specimens these colors become darker. The length of the stipe as well as the diameter of the cup vary greatly. The edges of the cup inroll when the specimen dries or has matured, completely closing the cup so that the interior is not visible. The spores are hyaline, generally ovate-elongate, uniformly 6 μ in diameter but varying considerably in length, mostly within the limits of 16–20 μ. The asci are clavate and hyaline, the majority measuring within the limits of 115–120 × 8–10 μ. The paraphyses are generally 10–20 μ longer than the asci and are 2 μ in diameter. The hairs are yellowish brown. All measurements were made from sections of mature apothecia mounted in a solution of one-half per cent sodium hydroxide.

This fungus approaches the measurements given for *D. calycina* and *D. fusco-sanguinea,* but differs from each in certain particulars. Its ascus and spore measurements agree with the original description of *D. calycina* but do not agree in respect to color of hairs and inrolled margin. On the other hand, the measurements for *D. calycina* as given by Hiley are much larger than those given in the original description for *D. calycina.* Since Hiley made an extensive investigation of this species, his measurements probably represent more nearly the true measurements of *D. calycina* in its parasitic form. If this view be accepted, the asci and spores of the white-pine fungus are too small to be those of the parasitic form of this species. Evidently the original description was based upon the saprophytic form of *Dasyscypha calycina* since specimens of this fungus on very small twigs of the larch which were collected in England in 1927 by Dr. Boyce of

the Office of Forest Pathology agree with the original description. In the color of the hairs and the inrolled character of the apothecia, this parasite agrees with *D. fusco-sanguinea,* but the sizes of the spores and asci are considerably larger than those given in the original description for this species. The following table gives a comparison of the asci, spores, paraphyses, and hairs of the four forms:

COMPARISON OF PARASITIC AND SAPROPHYTIC FORMS

Species	Asci (μ)	Spores (μ)	Paraphyses	Hairs
1. D. calycina (Schum.) Fckl. (probably saprophytic)	120 × 9	16–22 × 6–7	Hyaline, 2.5 μ	Hyaline
2. D. calycina fide Hiley (parasitic)	140–200 × 9–15	17–23 × 8–10	Hyaline, 2 μ	Hyaline
3. D. fusco-sanguinea Rehm. (probably saprophytic)	70 × 10	15 × 5	Hyaline, 2 μ	Reddish brown
4. D. sp. white-pine species (parasitic)	115–120 × 8–10	16–20 × 6	130–140 × 2 μ	Reddish brown

It is a fair inference from the study of this table and the facts previously stated that Number 4 bears to Number 3 the same relation that Number 2 bears to Number 1 and, notwithstanding the variations, the fungus on our western white pine may very properly be accepted as the parasitic form of *D. fusco-sanguinea.* This opinion is concurred in after independent investigations by Weir, Diehl and Dearness.[2]

DISTRIBUTION

The writer first had this disease called to his attention as white-pine blister rust in August, 1921, by a forest ranger on Fish Creek in Montana, at the western border of Glacier National Park. Since that time, its distribution has been found to be general in the white-pine region. The more important locations where it has been observed are given in this paper. As a rule, the most heavily infected areas occur at relatively high elevations, that is, 4500 to 6000 feet, on sites where white pine is near its altitudinal limits or otherwise on a site rather unfavorable for white-pine growth. However, it has been observed in young reproduction in some localities on fairly favorable white-pine sites, attacking about 20 per cent of the trees. At lower elevations in other parts of the region, it occurs only as an oc-

[2] The writer wishes to express his thanks to Dr. J. R. Weir and Mr. W. Diehl of the Office of Mycology and Disease Survey, United States Department of Agriculture, and to Professor John Dearness, Ontario, Canada, for examining specimens which were submitted to them.

casional infection on the smaller limbs, killing these, but causing no other evident damage.

The following are records of collections and observations of *Dasyscypha fusco-sanguinea* on white pine, *P. monticola*.

Montana:

Fish Creek, west edge of Glacier National Park, August, 1921. On trees about 10 feet tall. Fifty per cent of the trees infected. A few trees had dead tops and some were dead. Specimens on file at Forestry School, University of Idaho, Moscow.

Idaho:

Gold Creek, Section 17, Township 40 N., Range 3 E., Boise Meridian, Aug. 5, 1923. Large trunk cankers on trees 4 to 5 inches in diameter. Trees were evidently overcoming the disease.

Stoney Creek, Section 17, Township 41 N., Range 3 E., Boise Meridian, Aug. 10, 1923. Trees 1 to 7 inches in diameter, on about 2 acres, 75 per cent infected and several dead or dying. No. 2554.[3] (Fig. 1.)

Along trail Freezeout to Leu Boehls, Township 41 N., Range 2, 3, and 4 E., and Township 42 N., Range 3 and 4 E., Boise Meridian. Observed occasionally on branches of trees 4 to 8 inches in diameter. Aug. 11–12, 1923.

Upper Priest River, Section 24, Township 65 N., Range 5 W., Boise Meridian. July 30, 1924. Large basal, trunk cankers on trees 18 to 24 inches in diameter. Ascocarps found occasionally on the cankers. Elevation, 4000 feet; Section 18, Township 65 N., Range 4 W., Boise Meridian, July 30, 1924. Several acres in infected area. Trees 1 to 6 inches in diameter, about 75 per cent infected. Some tops and trees dead. Elevation, 4000–4500 feet. No. 2220 and 2594; Section 29, Township 65 N., Range 5 W., Boise Meridian, August, 1924. Occasional infections on young trees. Elevation, 4000 feet. Report and collection, P. S. Simcoe. No. 2595.

Shedroof Lookout, Section 36, Township 40 N., Range 45 E., Willamette Meridian, August 12, 1924. About 90 per cent of the pines under 15 feet high, infected. Seventy-five per cent killed. Several acres. Elevation, 4500 feet. Observed and reported by H. N. Putnam. No. 2598.

Upper Lionhead Creek, Sections 1 and 12, Township 62 N., Range 3 W., Boise Meridian, August, 1925. Trees, seedlings to poles. Nearly all infected. Observation by H. L. Whiting.

Green Mountain, Sections 1, 2, 11, 12, Township 40 N., Range 3 E., Boise Meridian. Trees 16 to 20 inches in diameter with large basal cankers. A belt about 100 yards wide on the south and southeast slope. Elevation, about 4500 feet. (Pl. XII, B.)

Bear Skull, St. Joe National Forest. September, 1927. Abundant all along the ridge. Trees 50 per cent infected or dead. Has general occurrence at 5500–6000 feet elevation in this region. Observation and collections by P. Rowe. No. 2600.

Yellow Dog Creek, Section 20, Township 23 N., Range 3 E., Boise Meridian, September, 1925. Spotted infection on trees 6 to 8 inches in diameter. Elevation, 3700 feet. Observed by J. R. Rodner.

[3] Numbers are for specimens in writer's herbarium.

Clarkia, Section 4, Township 42 N., Range 2 E., Boise Meridian, September, 1925. Infection occasional on branches of trees 6 to 8 inches in diameter. Elevation, 3200 feet. Observation by J. R. Rodner.

Quartz Creek, Sections 25 and 26, Township 58 N., Range 5 W., Boise Meridian, November 16, 1927. Occasional on trees 5 to 10 feet tall. Observed by C. C. Strong and P. S. Simcoe.

Washington:

North Baldy Mountain, Section 8, Township 36 N., Range 45 E., Williamette Meridian, July, 1925. About 50 acres on eastern exposure. Trees, seedlings to 4 inches in diameter. Nearly every tree infected and 50 to 75 per cent dead. Elevation, 4500 feet. Reported and collected by H. L. Whiting. No. 2597.

Mount Spokane, Northeast of Spokane, May 30, 1926. Fairly common and well distributed but not severe enough to cause much damage. Elevation, 5500 feet. Observation and collection by H. N. Putnam. No. 2599.

British Columbia:

Nelson, July 10, 1927. On many white pines 3–8 feet high. Many dead twigs. Observation and collection by J. W. Eastham. No. 2601.

Occasional limb infections have been observed in several other localities. It is needless, however, to record other smaller, infected areas which have been observed, since it is apparent from the above records that the parasitic form of *D. fusco-sanguinea* is quite generally distributed over the white-pine area of this region.

The writer has studied and collected specimens of Dasyscypha on conifers in the Northwest wherever they have been observed in the field, thinking that possibly *D. fusco-sanguinea* might occur on other trees or might be an ordinary saprophyte of this group which had found an especially congenial host in the white pine and thus had become an active parasite. In this connection the following collections have been made and are reported for the first time:

Dasyscypha agassizii (B. & C.) Sacc.; on *Pinus monticola* at Daisy Lake, B. C., June 21, 1924. No. 2064,[4] on *Pinus monticola* in Upper Priest River Valley, Idaho. July 31, 1924. No. 2221. Saprophytic and fruiting on dead branches.

Dasyscypha arida Phillips; on *Pinus contorta* Dougl., Warren, Idaho. August 24, 1920. No. 688; on *Abies lasiocarpa* Hook., Warren, Idaho. August 25, 1920. No. 698; on *Abies lasiocarpa*, Lookout Mt., Idaho. August 27, 1925. No. 2474; on *Picea engelmannii* Engelm., Upper Priest River Valley, Idaho. August 22, 1925. No. 2450; on *Pinus albicaulis* Engelm., Lookout Mt., Idaho. August 27, 1925. No. 2485. A common saprophyte, at the higher elevations, on dead branches and trunks of dead trees which have fallen into moist places, the species has a rather misleading name, since in this region it has been observed in such damp situations.

Dasyscypha calyciformis; on *Larix occidentalis*, Priest River, Idaho. May 12, 1920. No. 1412; on *Abies grandis* Lindl., Bungalow, Idaho. June 22, 1923. No.

[4] Numbers refer to collection or specimen number in writer's herbarium. Determinations for most of the collections have been checked by John Dearness, London, Ontario, Canada.

2297; on *Pinus monticola* Dougl., Cheekye, B. C., May 9, 1927. No. 2602. This species is ordinarily a common saprophyte, occurring on dead branches that still remain on the tree. In some cases, it appears to be a very weak parasite but, generally, there is some evidence that the attacked branch is in a weakened condition due to dense shade or injury of some kind. Occasional on blister-rust cankers.

DESCRIPTION OF THE DISEASE

The method of attack appears to be through the needles or needle fascicles, since the early initial infection areas are found as shrunken circular spots around the fascicle of the needle (Pl. XII, C). The bark shows distinct depressions for each year's growth (Pl. XII, A) due to the shrinking and drying out of the bark, so that the rate of spread of the disease each year may easily be determined. The rate of growth of these areas gives an indication of the resistance of the tree to the attack of the fungus. Very often there is a distinct cracking of the bark at the end of the season's growth. This is especially true if the fungus experiences difficulty in making progressive growth in the tree (Pl. XII, E). Occasionally this cracking at the point of farthest advance of the fungus may show signs of the formation of callus. The distinct separation by cracking of the growth of different years and the formation of callus becomes more evident as the diameter of the tree increases. On very young twigs the fungus will soon kill the branch, and the bark shows the general cracked condition (Pl. XII, D). The fungus appears to develop about twice as fast longitudinally as it does laterally. It apparently is not able to girdle trees over four inches in diameter. Field observations indicate that the larger the limb or trunk attacked, the less probable it is that the fungus will be able to girdle it.

The typical orange yellow apothecia appear on the area attacked the previous year; in other words, the fungus fruits on the bark in which its mycelium is in the second year of its growth. In the case of the smaller branches, girdled and killed the first year, no apothecia may appear, or, they may appear only on the first part of the canker, indicating that the fungus is primarily a parasite. Fruiting may occur on the several years of growth of the canker and appears to continue on a particular year's growth until the bark dries up and cracks, indicating that the tissue in the area is dead. This dead bark adheres quite firmly to the wood underneath. Due to cutting off of food supply from above there is generally a swelling of the tree above the point of attack. This swelling is especially prominent at the nodes when the main trunk is attacked through one of the nodal branches or just below this point. There is generally an abundant exudation of pitch from cankers. In many cases, especially those of trees over four inches in diameter which have trunk cankers, the fungus seems unable to kill the tissue in the infected area, but continues to grow there year after year

(Pl. XII, A). Evidently the fungus grows in the meristematic tissue, causing a swelling of each year's woody tissue, thus causing a long, oblong swelling in the wood itself. An examination of the wood in these oblong swellings reveals distinct resinous, blackish brown layers in the spring wood, typical for such parasites. In such cases it appears that the fungous growth evidently causes the swelling of both the woody and bark tissue.

There appears to be a limit to the size of the trees killed by this disease or on which the disease can make definite lateral and longitudinal progress. On large trees a deep-seated canker is formed similar to those described for the larch canker. Many trees 6 to 8 inches in diameter have been observed with these deep-seated cankers. Apparently in these cases the fungus in the canker had been unable to spread laterally or longitudinally to any extent, but continued to grow in the meristematic tissue that developed in each new year's growth, killed it, and thus prevented the tree from healing over the wound. Several trees 4 to 8 inches in diameter have been observed, so reduced in mechanical strength at the point of the canker that some had bent over at a right angle toward the cankered side. Figure 1 shows the enlargement on such trees opposite the canker and the partial consequent weakening of the tree.

Trees 10 to 30 inches in diameter have been found with deep-seated cankers about their bases but they evidently had been able to overcome the attack and continue growth, so that the fungus had done little more than distort the tree at the canker. Plate XII, B, shows a typical instance. In these cases the fungus appears to continue to grow and to kill the meristematic tissue at the edge of the canker, thus preventing the tree from healing it over. This causes a shrunken, distorted area. The cankers occur on no particular side of the tree nor at a regular height among any group of trees. No cankers, however, have been observed more than eight feet from the ground in the case of large trees. Large scales of dead bark are found near the edges of these cankers, showing that the disease evidently counteracted the efforts of the tree to heal over the wounds. When the bark is removed, a canker several inches deep is revealed. It is generally elliptical, a few inches in diameter at the bottom of the canker, and several times as large in diameter at the outer edge. The depth of the canker, of course, depends on the diameter of the tree. The canker resembles a minute amphitheater. In all cases examined, it has been observed to extend to within four or five annual rings of the center of the tree, indicating early infection of the tree.

Two areas where practically all of the white pines were thus cankered have been observed on trees 10 to 30 inches in diameter; one on Green Mountain, about 12 miles east of Elk River, Idaho, the other on the west slope of the Upper Priest River Valley, Idaho, in the Kaniksu National

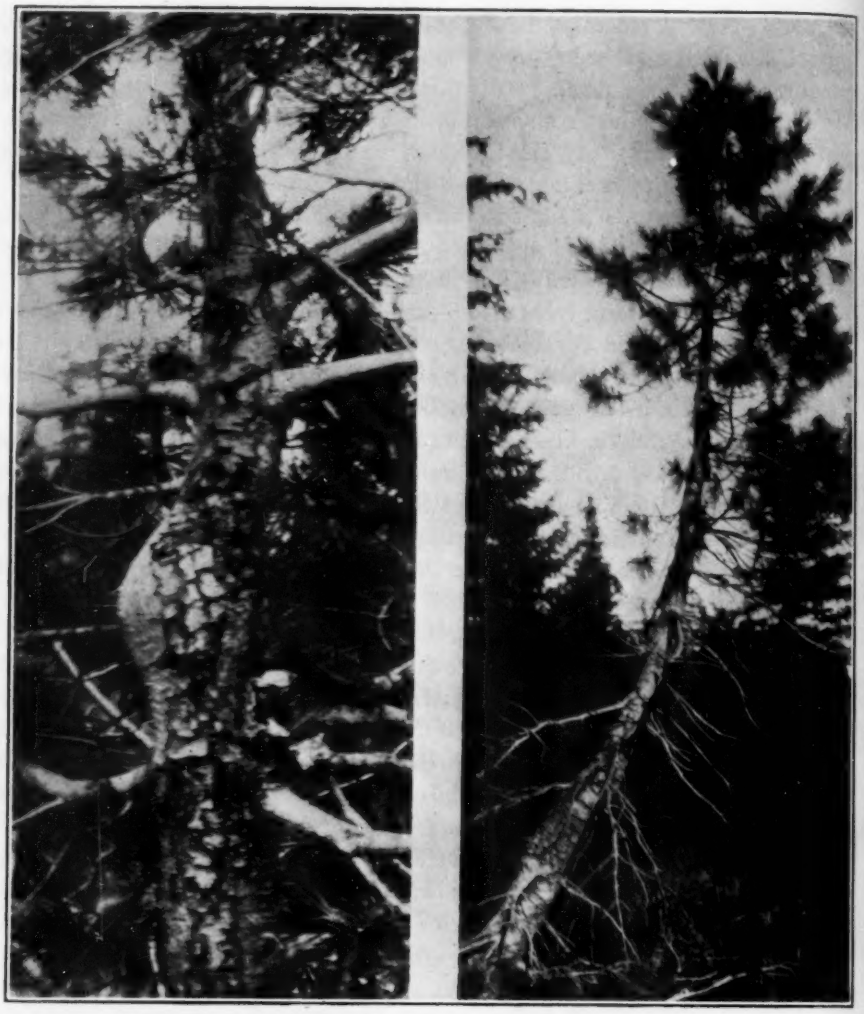


FIG. 1. A, shows a white-pine tree distorted by the Dasyscypha canker. The enlarged portion is directly opposite the canker; B, the enlarged bole at base of tree and limbs being killed by Dasyscypha. No canker occurred on this tree but abundant apothecia developed on the bole and limbs.

Forest. These areas are at elevations of about 4500 feet and hence are near the upper limits of the white pine. Ascocarps of the fungus are not commonly found on these large cankers. On the Upper Priest River area, however, ascocarps were found occasionally. Since these cankers are so similar to those described for the larch and, since similar fruiting bodies have been found on them, it is reasonable to believe that they are caused by the fungus.

Indeed, they coincide quite closely with those described by Hiley (2) on larch. The infected woody tissue, whether the tree be large or small, is impregnated with pitch, making it difficult or impossible to remove sections with a hand axe. In the trees attacked, the butt log, or at least the first eight feet, will be lost entirely.

COMPARISON WITH WHITE-PINE BLISTER RUST

The white-pine-blister-rust canker produced on older trees is very distinct from the canker produced by the parasite, *Dasyscypha fusco-sanguinea*. The old blister-rust canker is characterized by broken, curled, cracked bark on the surface of the tree trunk. The Dasyscypha canker is a deep-seated, distorted cavity, extending nearly to the center of the tree, the cavity appearing as a minute amphitheater. In both cases there is a considerable development of resin in the wood and exudations of it from the cankers stream down the trunk. The typical aecia of blister-rust will generally develop in the bark which has not yet been killed, and the extension of the mycelium into the living bark produces a distinct brownish discoloration. The apothecia of the Dasyscypha on the cankers on older trees are very infrequent and occur on the very edge or slightly inside the edge of the canker.

There is much more similarity in the gross characters of the two cankers as they occur on the younger trees. The incipient stages of the Dasyscypha infection produce a brownish, shrunken condition of the bark. Blister rust causes an orange discoloration of the bark, generally accompanied by a slight swelling of the whole area. This is the stage when the greatest care must be exercised if the two are not to be confused.

After one year's growth, the two may be easily distinguished. Each year's growth of the Dasyscypha is definitely indicated by a marginal ring either of broken bark or by a slight raised area at the edge of the year's growth, due to the fact that the bark at the edge did not shrink as much as toward the center of the canker. Further, the apothecia appear abundantly on the past year's growth. On the blister-rust canker the yellowish brown pycnial spots will be found on the area where the fungus has been present for more than one year and, soon after, the bark is broken by the extruding aecia.

There may be an abundant flow of resin in both cases when young trees are attacked to such an extent that the differences mentioned above are somewhat obscured. By a careful examination of the infected area, however, by one who knows the two diseases, no real difficulty will be encountered in determining which organism is present.

SUMMARY

A common canker of western white pine, *Pinus monticola*, which is being confused with cankers of white-pine blister-rust, is here reported for the first time.

The canker is generally distributed over the white-pine region of the Inland Empire.

The cause of the canker is believed to be a parasitic form of *Dasyscypha fusco-sanguinea*.

The canker is very similar to the larch canker caused by *D. calycina*.

It has many gross characteristics similar to those of the white-pine blister rust.

It is a disease of secondary importance causing considerable damage in white-pine sites favorable to its development.

PLANT QUARANTINE AND CONTROL ADMINISTRATION,
UNITED STATES DEPARTMENT OF AGRICULTURE,
SPOKANE, WASHINGTON.

LITERATURE CITED

1. ANDERSON, A. P. *Dasyscypha resinaria* causing canker growth on *Abies balsamea* in Minnesota. Torrey Bot. Club Bul. **29**: 23–34. 1902.
2. HILEY, W. E. The fungal diseases of the common larch. 204 pp. Clarendon Press, Oxford. 1919.
3. JELSTRUP, HENRIK. Innberetning fra statsmykolog Ivar Jørstad om skydommer på skogtraerne i årene, 1920–1925. Innberetning om det Norske skogvesen og endel under Landbruksdepartmentets Skogkontor hørende anliggender for året 1925. Kap. 85–95. Skogdirektoren, Oslo. 1926.
4. SACCARDO, P. A. Sylloge Fungorum. **8**: 1143 pp. 1889.
5. SPAULDING, P., and P. V. SIGGERS. The European larch canker in America. Science N. S. **66**: 480–481. 1927.
6. TUBEUF, K. VON. Diseases of plants induced by cryptogamic parasites. 598 pp. Longmans Green and Co., N. Y. 1897.
7. UNDERWOOD, L. M., and F. S. EARLE. A preliminary list of Alabama fungi. Ala. Agr. Exp. Sta. Bul. 80: 113–283. 1897.
8. WAGNER, G. Beiträge zur Kenntniss der Pflanzenparasiten. Zeitschr. f. Pflanzenkrank. **6**: 321–323. 1896.

PLATE XII

A. Apothecia, rings of annual growth of the fungus, and swollen node.

B. A white-pine tree with two Dasyscypha cankers on the trunk. Typical of infection on Green Mountain east of Elk River, Idaho.

C. Typical infection spots at needle fascicles on a small branch.

D. Young branch dying from Dasyscypha attack. Brownish needles removed in order to show cankered bark.

E. Formation of callus at edge of annual growth. The extent and growth of the fungus, however, are not strictly confined to the swollen portion of the branch.

A
B
C
D
E

RHIZOCTONOSIS OF LOBELIA[1]

S. C. Teng

In the summer of 1927, Professor H. H. Whetzel called the writer's attention to a serious trouble of the double blue Lobelia (*L. erinus* L.) in the greenhouse of the Floriculture Department at Cornell University and suggested that an investigation of the disease be undertaken. It is hoped that the observations and experiments here reported may serve to call attention to a disease probably responsible for much of the difficulty commonly experienced in growing the double Lobelia in the greenhouses. What is probably the same disease has been reported by Rant[2] on *L. erinus* growing in his garden in Java.

SYMPTOMS AND ETIOLOGY

The characteristic symptoms of the disease under humid conditions in the greenhouse are the hydrolysis and yellowing of the lower leaves and the formation of necrotic lesions at the base of the stem. There follows a rotting of the affected organs and the consequent wilting and death of the parts above (Pl. XIII, A). While rotting, the plants often show a thread-blight aspect, the mycelium of the fungus growing all over the plant. Sometimes abundant aerial roots are formed just above the lesions at the base of the stems. After the death of the tops, new shoots may develop from the crowns. In some cases the plants become stunted and exhibit an abnormally dark green foliage.

A Rhizoctonia was isolated from the diseased Lobelia plants. Its characters agree in general with those described for *Rhizoctonia solani* Kühn. In comparison with a strain of *R. solani,* which causes the wire stem of cabbage, and another isolated from a potato tuber, the Lobelia strain has a somewhat higher optimum temperature for growth, about 27° C. The test was carried out in triplicate on both Czapek's agar and potato-dextrose agar. The effect of temperature on the growth of these strains was measured by the increase in diameter of thalli in 24 hours. The optimum growth temperature for the other two strains is about 24° to 25° C. When grown on the same culture medium and at temperatures optimum for each, the Lobelia fungus grew about twice as rapidly as the organism from potato. It also outgrew the cabbage Rhizoctonia by a wide margin, the ratio being approximately 3:2. Two days after planting on potato-dextrose agar in petri dishes at 27° C., the mycelium was found to cover the whole plate; and five days after planting, the formation of sclerotia became evident. The sclerotia were larger

[1] The writer acknowledges his indebtedness to Professors H. H. Whetzel and H. E. Thomas for their valuable suggestions and encouragement in connection with this investigation.

[2] Duggar, B. M. Rhizoctonia Solani in relation to the "Mopopilz" and the "Vermehrungspilz." Ann. Missouri Bot. Gard. 3: 1–10 (See p. 8). 1916.

in size and fewer in number than those of the other two strains (Pl. XIII, B, b).

The fungus makes more rapid growth and produces more sclerotia on Czapek's agar than on potato-dextrose and oat agars, (Pl. XIII, C). The difference between the growths on the two latter agars is not striking. The mycelium in culture is rather appressed. That on Czapek's agar is of a more or less fawn color; while on potato-dextrose and oat agars, it is pale ochraceous buff and light buff (Ridgway), respectively. An important characteristic of the fungus is that it forms one large sclerotium, often 1 to 1.5 cm. across, at the center of the plate and smaller sclerotia in a zone surrounding the center and again at the margin of the plate. The sclerotia are usually rather wooly and vary in color from wood brown to cinnamon brown. Immature sclerotia usually exhibit prominent drops of brownish liquid on their surfaces. The description of the cultural characters of the fungus is based on 16-day old cultures incubated at 27° C.

Late in the summer a partially yellowed leaf near the base of one of the diseased plants was found covered to the base of the petiole with a loose mat of ashy gray mycelium. Freehand sections showed that this weft of mycelium consisted of basidia arising from typical Rhizoctonia hyphae. The writer failed to get cultures of the Rhizoctonia from the basidiospores. However, a portion of the material was preserved in alcohol, and slides were prepared from this by the ordinary paraffin method. These clearly showed the fruiting stage of the fungus which agrees closely with the description of *Corticium vagum* B. & C. as given by Burt.[3]

PATHOGENICITY

The pathogenicity of the fungus was readily established by inoculation experiments upon double blue Lobelia. All inoculations in this investigation were made by the use of steamed wheat kernels bearing a heavy growth of the fungus and placed about the base of the plant below the surface of the soil. In every case the soil or sand was steam sterilized. All experiments were carried out in a greenhouse having an average temperature of 70° F. The soil used was a silty loam.

The first experiment was made on full-grown plants in pots. After inoculation, they were covered with bell jars and generously watered. The plants showed evidences of infection in two weeks. The fungus was reisolated from the diseased plants, and about a month after death and the removal of the diseased plant, it was also recovered from the soil in which the plants had grown. The method of isolation from the soil was as follows: The soil was put into a sterile preparation dish and watered with sterile water. Seeds of cucumber, which had previously been found to be suscepti-

[3] Burt, E. A. The Thelephoraceae of North America. XV. Corticium. Ann. Missouri Bot. Gard. 13: 173–354. 1926.

ble to this fungus, were sown in this soil. The seed was superficially sterilized in calcium hypochlorite before sowing. The seedlings promptly became infected and the fungus was isolated from these in the usual manner.

In a further experiment, cuttings from apparently healthy Lobelia plants were planted in sand planted with the fungus. Within a week, 39 of the 40 cuttings inoculated were prostrate, while of the 60 cuttings in the control flat, 58 lived. The two that died in the check flat showed no evidence of Rhizoctonia.

After having established the pathogenicity of the fungus on the double blue Lobelia, its pathogenicity to four other cultivated Lobelias was tested. Full-grown plants as well as seedlings were used. The former were inoculated by placing infested wheat kernels in the soil, as before described. They were then covered with bell jars. Seedlings were inoculated at the time of transplanting into pots. It was found that *L. erinus* var. *gracilis* is resistant to the attack of the fungus. *L. erinus* var. *speciosa* (White Compact) and *L. ramosa* Benth. succumb when the roots are wounded and the plants put under abnormally moist conditions. Some of the cardinal flowers (*L. cardinalis* L.) became distinctly stunted.

The susceptibility of certain vegetables to the Lobelia Rhizoctonia was also tested. Infested wheat kernels were mixed with the soil just before planting the seed. Severe damping-off was produced by the fungus on cucumber seedlings (Early Fortune). On seedlings of red kidney bean considerable damping-off and stem rot were produced. The fungus was reisolated from diseased bean and cucumber plants. Cabbage (Copenhagen Market) and beets (Crosby's Egyptian) showed moderate susceptibility, while pepper (Ruby King) was found to be resistant in these tests. On both beet and cabbage seedlings a wire stem effect was produced.

CONTROL

Laboratory experiments to test the fungicidal effect on the Lobelia strain of Rhizoctonia were made with sulphur (Niagara Kolodust), aluminum sulphate, copper carbonate, mercuric chloride, Uspulun, Bayer Special II–6 (6 per cent nitrophenol mercury), Bayer Special 144 (4 per cent nitrophenol mercury), and Bayer Special 154 (mixture of 6 per cent chlorophenal and 8.2 per cent nitrophenol mercury). These tests were made by dusing petri-dish cultures of the fungus with the above-named chemicals, and taking the growth of the fungus after dusting as a criterion of the effectiveness of the chemicals. All the mercury compounds tried were found effective. There was no increase in the diameter of the thallus after dusting. Copper carbonate was not so effective, aluminum sulphate still less, and sulphur the least.

Dusting the soil in which double blue Lobelia plants were growing in the greenhouse with various mercury compounds was then tried. Where the dust came in direct contact with the foliage of the plants chemical injury

resulted. This difficulty was overcome by using the chemicals in solution or in suspension in water. Mercuric chloride, being much cheaper than the organic mercury compounds, was selected for further experimentation. Wiant[4] reports that aluminum sulphate is effective in controlling *Rhizoctonia solani* in beds of *Pinus resinosa* Torr. This material being exceedingly cheap was, therefore, also selected for further studies. It may be applied in either dry or liquid form.

Cabbage and cucumber, being readily grown and also susceptible to the attack of the Lobelia Rhizoctonia, were used in an experiment to determine the dosage of mercuric chloride and of aluminum sulphate necessary to check the activity of the fungus. It was found that three-tenths of a gram of mercuric chloride in a liter of water applied at the time of sowing to each 1½ square feet of space is effective. Five-tenths of a gram of mercuric chloride caused slight and higher concentrations distinct chemical injury to the plants. Aluminum sulphate was found ineffective in controlling the fungus. At a concentration of twenty grams to each 1½ square feet of space, it was evidently injurious to the plants. It was found that double Lobelia plants can withstand five-tenths of a gram of mercuric chloride to each 1½ square feet of space without evident injury. Therefore, for heavily infested soil, one tablet of mercuric chloride in a liter of water to an ordinary 12″ x 18″ flat should be a safe dosage. For slightly infested soil or "unclean" sand one tablet in two liters of water, applied at the rate of one liter to a flat, probably will suffice.

The fungus easily gains ingress to its suscept below the surface of the soil, especially at the time when the latter is propagated in the form of cuttings. Unless clean sand and soil are used, the application of mercuric chloride should be made at the time of propagation and of transplanting. The use of cuttings from healthy plants for propagation is to be recommended. Various observations seem to indicate that humid conditions favor the development of the disease. Any cultural method that will reduce this factor to a feasible minimum will also help to lessen the ravages of the disease.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, NEW YORK.

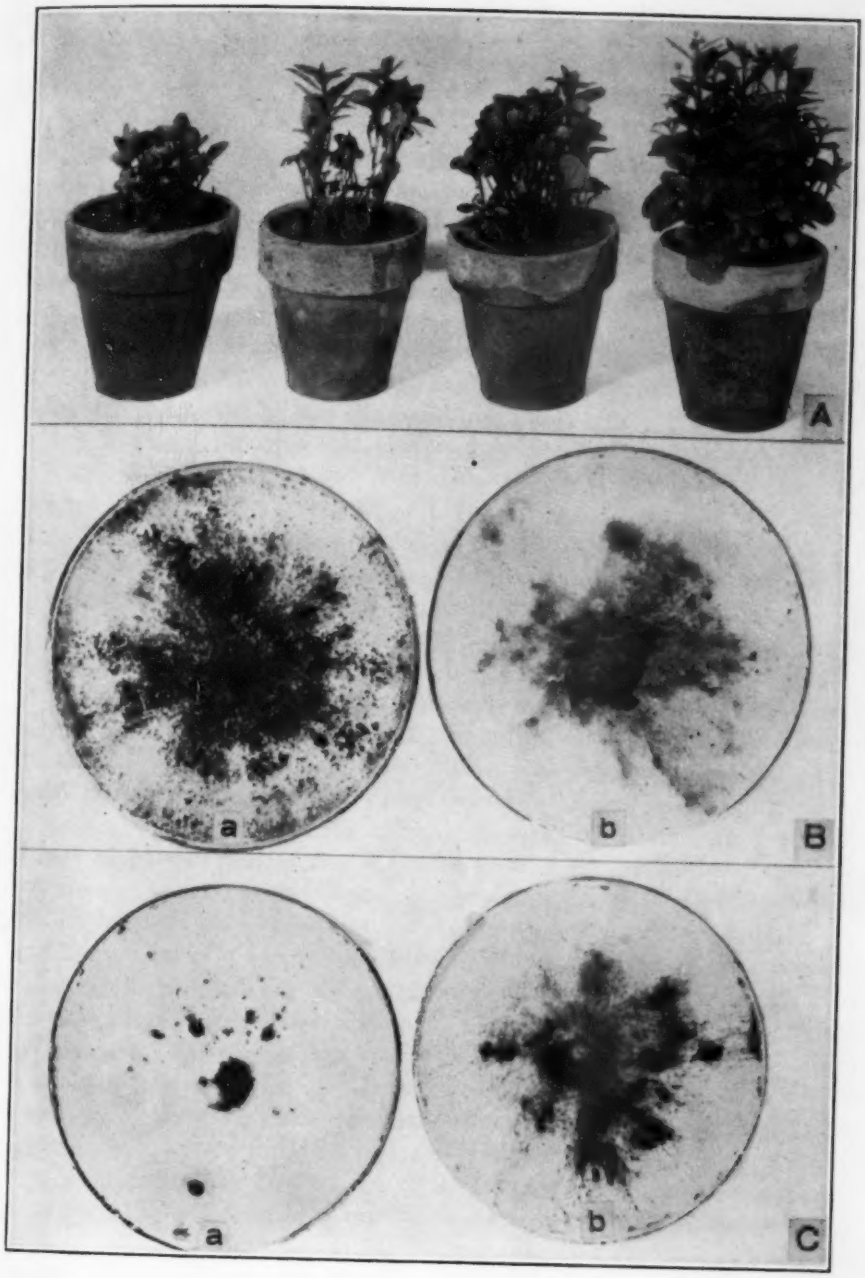
PLATE XIII

A. Showing symptoms of the disease on potted plants of double blue Lobelia (*L. erinus*). The plant on the extreme right is healthy.

B. Showing differences in cultural characters of the potato strain (a) and the Lobelia strain (b) of Rhizoctonia on Czapek's agar. Cultures 16 days old grown at 25° C.

C. Showing cultural characters of the Lobelia strain of Rhizoctonia grown on oat agar (a) and on Czapek's agar (b) at 27° C.; cultures 16 days old. This strain grows more vigorously and produced more sclerotia on Czapek's agar.

[4] Wiant, J. S. Soil treatments for the control of damping-off in coniferous seedbeds. (Abst.). Phytopath. 17: 51–52. 1927.


A
a
b
B
a
b
C

FALSE SMUT OF MAIZE, USTILAGINOIDEA

R. J. Haskell and W. W. Diehl

Early in September, 1925, C. W. Edgerton, of the Louisiana Agricultural Experiment Station, transmitted some corn tassels bearing a fungus that had the appearance of the common smut, *Ustilago zeae* (Beckm.) Ung. A closer examination, however, showed that the various excrescences on the inflorescence were unlike those caused by corn smut, being decidedly different in form, size, color, and texture. An examination of the spores, which completely covered the surface in a smutty mass, showed that the fungus was a species of Ustilaginoidea. This material had been gathered by J. G. Richard, County Agent, at Houma, Louisiana, in August, 1925, and constituted the first report of this disease to the Plant Disease Survey. Dr. Edgerton, however, reported that this was not the first time he had observed it. The following year better and more abundant material was received through the Office of Foreign Plant Introduction. It had been obtained by H. Johansen during August, 1926, in the Plant Introduction Garden at Summit in the Canal Zone (Panama). Although two preliminary reports (4 and 5) concerning these two occurrences seem to be the only published accounts of Ustilaginoidea on Zea, the incidence of fungi of this genus on related hosts is not uncommon, especially in the Tropics.

The writers have not seen this disease in the field and, so, can describe it only from the dried specimens. The principal features thus far observed are the characteristic growths on the staminate inflorescence (Plate XIV, A and B) which, when mature, range from 4 to 15 mm. in diameter. These growths are very irregular in shape although, in general, they may be classed as subspherical. They are sclerotia of a fungus and not tumorous host tissue. Usually there is but one sclerotium on a spikelet although those on neighboring spikelets sometimes fuse to form compound sclerotia.

The surface of a mature sclerotium is much roughened, olive-green to almost black, and of a velvety appearance. The younger sclerotia are various tints of yellowish brown shading into green. When one cuts across a sclerotium the interior is seen to be made up for the most part of white, sclerotioid, fungous tissue including scattered host fragments. The surface layer is very different in texture and color from the interior tissue. The color shades from the white of the interior to yellow, then through brown to olive green and finally to the black of the surface. The extreme outer layer is found to be made up of a mass of mature spores with detached fertile hyphae. As the spores mature they become progressively darker, hence this surface is darker than the portion made up of younger spores and fertile hyphae directly beneath. This spore-bearing stratum covers the entire

periphery of the sclerotium to a depth of 50–200 μ. The fertile hyphae are closely packed at its base, becoming more loosely and irregularly arranged toward the exterior. They are septate, occasionally branched, yellowish green in color (by transmitted light) and 2–3 μ in diameter. The spores are borne on very short, sterigma-like projections from the walls of these hyphae and are spherical to ovate, 4–7 μ, usually 6 μ, in diameter, rarely larger. Immature spores are nearly smooth but with maturity the epispore is characterized by prominent pointed warts which, together with adhering matter, give an indistinct outline to the spore (Fig. 1).

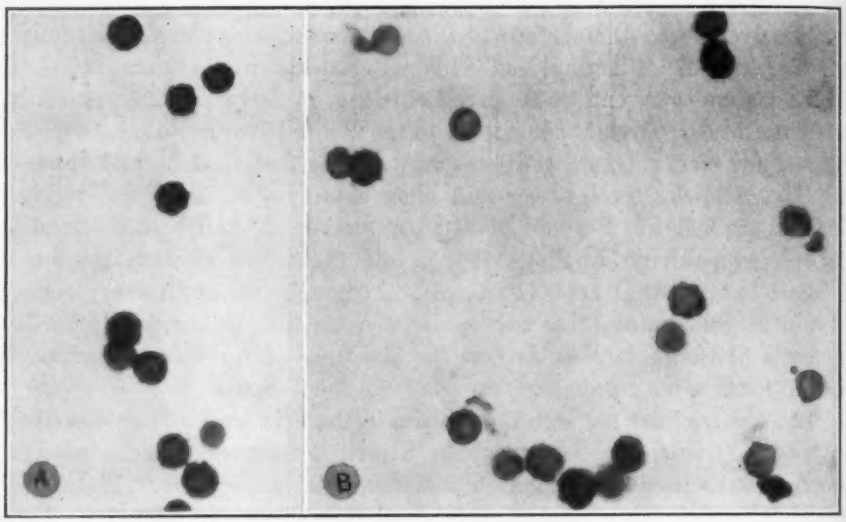


FIG. 1. Spores of Ustilaginoidea (×800), from (A) corn, Canal Zone; (B) rice, Crowley, Louisiana, 1921, collected by J. Mitchell Jenkins. (Photographed by J. F. Brewer.)

The identity of the species under consideration presents certain difficulties since the specific limitations and host relations are very imperfectly known. As far as specimens and records are available to the writers the hosts seem to be restricted to the family Gramineae. The fungus on corn appears to be distinct from the other species forming sclerotia exterior to the host tissues, but it is similar in measurable, microscopic characters to certain species having sclerotial development within the caryopsis. Of these latter it seems to resemble most closely the false smut of rice, *Ustilaginoidea virens* (Cke.) Tak., to which it is tentatively referred.

The false or green smut of rice is known to occur in all the great rice-growing areas except in Italy and Egypt. Butler (2 and 3) has discussed its distribution and significance. In the United States it has been recorded

with the evidence of specimens from Louisiana, only. There is, however, in the files of the Plant Disease Survey a report, unaccompanied by specimens, of its observation in Georgia, August 20, 1918, by J. B. Berry. In Louisiana it has been known since about 1900 (6, p. 505), when it was noticed as a curiosity by a few planters. Since that time it has been found occasionally but in no case has it been recorded as at all abundant.

The fungus and its effect on the rice plant have been described by various authors, notably by Butler (*l.c.*). On this host it develops within the grain, forming a sclerotium surrounded by a conidial layer. In its growth it bursts out between the closely applied glumes as a more or less spherical body (Plate XIV, C and D) which is orange tan to yellowish brown when young, becoming brownish green to almost black with age. In every detail so far observed, except for the somewhat smaller size of the sclerotium and its development mainly within the host tissue, *Ustilaginoidea virens* on rice resembles the form on corn.

Of the species of Ustilaginoidea on other hosts, those described with spores of similar dimensions are lacking in sclerotial development comparable with that discussed above and, until further evidence of relationship is available, may be omitted from consideration.

Ustilaginoidea virens has attracted only scant attention from the economic standpoint. One writer (8), however, ventures the opinion that the sclerotia may not be the sole evidence of injury to rice and that other losses such as spikelet sterility may be partly due to this cause. In the Philippines it is said (1 and 7) to cause an appreciable loss during some years of damp weather. In Louisiana it is not considered to be an economic factor.

On corn the disease probably will be even less injurious than on rice. As far as we know, it attacks only the tassels and results in little damage except a partial decrease in pollen production. There is a possibility that it may attack other parts of the plant, in which case the damage would naturally be more serious. It is an additional disease of one of our important crops and as such it should be watched. It is realized that owing to its superficial resemblance to corn smut it may easily be overlooked. When observations are made or specimens collected it is hoped that they may be reported to the Office of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.

SUMMARY

A new disease of *Zea mays* is reported from Louisiana and the Canal Zone. Excrescences somewhat resembling those of common smut occur on the corn tassels. The causal fungus is an Ustilaginoidea and is tentatively referred to as *Ustilaginoidea virens* (Cke.) Tak.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

LITERATURE CITED

1. BAKER, C. F. *Oryza sativa.* Philippine Agr. and For. **5**: 75. 1916.
2. BUTLER, E. J. False smut. Pusa Agr. Res. Inst. Bul. **34**: 30–34. 1913.
3. ————————. Rice. False smut. Fungi and disease in plants: 228–230. 1918.
4. HASKELL, R. J. Green smut caused by *Ustilaginoidea* spp. U. S. Dept. Agr., Bur. Pl. Ind. Pl. Dis. Reptr. Suppl. 48: 357. 1928.
5. ————————. A green or false smut of corn caused by *Ustilaginoidea.* U. S. Dept. Agr., Bur. Pl. Ind. Pl. Dis. Reptr. 10: 132–133. 1926.
6. ORTON, W. A. Plant diseases of 1906. Yearbook U. S. Dept. Agr. **1906**: 499–508. 1907.
7. REINKING, O. A. False smut or lump smut. Phil. Jour. Sci. **13**: 226. 1918.
8. VINCENS, F. *Ustilaginoidea virens* (Cke.) Tak. Bull. Agr. l'Inst. Sci. de Saigon **3**: 311. 1921. (Abstract in Rev. Appl. Myc. **1**: 157–160. 1922.)

EXPLANATION OF PLATE XIV

A. Ustilaginoidea on corn ×½ (material from Canal Zone). B. Detail of above ×12½. C. Ustilaginoidea on rice ×1 (material from Crowley, La.). D. Detail of above ×4. (Photographed by M. L. F. Foubert.)

A
C
B
D

A METHOD OF TESTING THE KEEPING QUALITY OF CERTAIN SMALL FRUITS

NEIL E. STEVENS

Breeding for disease resistance is almost a fad among plant pathologists and it is surely better, where practicable, to grow plants which will be largely free from disease than those which will produce profitable crops only with the aid of sprays or dusts. The interest in disease resistance, however, seems rarely to have extended even among the fruits, to breeding for resistance to decay in transit, on the market or in storage, that is, for keeping quality. Yet keeping quality has often been a prime factor in determining the commercial importance of a variety, as is evidenced by the Ben Davis apple, Elberta peach, Klondyke strawberry, and Howes cranberry.

The importance of keeping quality is perhaps widely recognized by breeders of small fruits and might very generally be considered as a factor in selecting varieties for propagation if some simple method for testing this character applicable to small quantities of fruit was generally available. The method described in the present paper has proved reasonably successful as a means of testing cranberries and blueberries.

METHOD

The method is simple. Pint samples from a given variety or lot are held in an electric incubator for one week at a temperature of 30 to 32° C. The fruit is then sorted. Cranberries are usually sorted into two lots, sound and rotten. Blueberries are usually sorted into four lots, perfect berries, shriveled (even slightly), soft, and moldy.

RESULTS

In the work with cranberries, attention was directed chiefly toward determining the relative keeping quality of different lots of standard varieties grown on various bogs through a series of years. For most varieties of cranberries when grown in Massachusetts or Wisconsin, the incubator test does seem to indicate, with a high degree of accuracy, the keeping quality of the different lots at ordinary storage and shipping temperatures. The chief exception seems to be the Howes which, in these States, is very generally affected by end rot caused by a fungus which will grow at temperatures only a little above 0° C. So far, we have not found this method so satisfactory for cranberries grown in New Jersey due, apparently, to the prevalence of high temperature fungi in that State.

For the past three years we have been using the same method as a means of testing the keeping quality of the new varieties of blueberries which are being planted commercially in New Jersey. As is generally known, these new varieties are either selected wild plants of *Vaccinium corymbosum* or crosses between plants of this species, or between this and other species, which have been developed and named by Dr. Frederick V. Coville. The past three years' work indicates that the varieties behave with a fair degree of consistency year after year and that their behavior in the incubator is a fairly reliable index of their behavior at common storage temperatures.

TABLE 1.—*Comparative keeping quality of blueberry varieties as indicated by average percentage of perfect berries*

One week at 30° C.		*Four weeks in cold storage*	
Rubel	20	Jersey	35
Jersey	18	Rubel	35
Pioneer	13	Grover	21
Grover	12	Pioneer	18
Cabot	9	Cabot	11

The work of the past year seems to show that for the five varieties that were tested in 1928 there is a fairly constant relation between the keeping quality of the different varieties at 30° C. and in commercial cold storage, as shown by table 1 which gives the average keeping quality throughout the season expressed as percentage of sound berries. This does not, of course, indicate the amount of edible berries, as even slightly withered berries are eliminated from the count, but the relative standing of varieties is not very different even if the total number of edible berries is considered, as is shown in table 2, in which perfect, shriveled, and spoiled berries were

TABLE 2.—*Comparative keeping quality of blueberry varieties according to a weighted scale*[1]

One week at 30° C.		*Four weeks in commercial cold storage*	
Jersey	60	Jersey	72
Rubel	41	Rubel	67
Pioneer	24	Grover	65
Grover	20	Pioneer	53
Cabot	17	Cabot	36

[1] The values in this table were obtained by assigning a value of 1 to each perfect berry, a value of 2/3 to each shriveled berry and −1 to the soft or moldy berries. It makes little difference in the relative standing of the varieties what values are chosen.

all considered according to a weighted scale. It will be noted that Rubel and Jersey occupy a high position, Grover and Pioneer are intermediate, while Cabot is lowest in all the columns.

DISCUSSION

It is, of course, not to be expected that this close correspondence in behavior at high and low temperatures will be found among all small fruits or that it will necessarily hold for all the new varieties of blueberries that are constantly being developed. Overholser[2] has recently called attention to the fact that "the better the intrinsic keeping quality at room temperatures, of the species of fruit stored the greater the proportional relative delay in senescence brought about by cold temperatures." He further points out "that certain varieties within a given species kept relatively long at 18° to 24° C. and comparatively short periods of time at 0° C. Furthermore, with other varieties of the same species the reverse was true."

In spite of this undoubted difference between the varieties of some species, there may be sufficient correlation in the behavior of others at high and at lower temperatures for one to serve as a working index of the other. At least the apparent success of the method in this case seems to justify its further use and study.

To consider keeping quality as an important character in breeding does not mean a neglect of eating quality. It will undoubtedly be possible to combine in the same berry high flavor and good keeping quality. In the past, by a process of natural or commercial selection, those varieties have been most largely planted which showed good keeping quality regardless of their eating quality. The result has often been extensive plantings of varieties of distinctly inferior eating quality. An example of this is the Klondyke strawberry which occupies about one-fourth of the commercial acreage of strawberries in the country. This variety, incidentally, is said to have been developed as a result of selection for keeping quality. The Bureau of Plant Industry already has in its breeding plots at Bell, Md., varieties far superior to it in eating quality which seem, from our preliminary tests, to be superior in keeping quality also.

As regards the blueberry, the possibility of putting on the market a variety which did not possess good eating quality has never been considered. All the varieties used in these tests are of high, although not equal, eating quality. We are merely trying to determine which of these unusual fruits has the best keeping quality. The work of testing varieties of blueberries will be continued, and we plan to try a modification of this method on strawberries.

[2] Overholser, E. L. Cold storage versus room temperatures on keeping qualities of fruits. Science 66: 660–661. 1927.

SUMMARY

Holding pint samples of blueberries and cranberries at 30° C. for one week apparently gives a fairly reliable index of their keeping quality at lower temperatures.

In view of the recognized importance of keeping quality in determining commercial importance of varieties of fruits, it is suggested that this method is worthy of further trial by breeders of small fruits.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

BLUE-STAINING FUNGI FOUND IN THE UNITED STATES

CAROLINE T. RUMBOLD

This article presents the results of an investigation conducted by the Bureau of Plant Industry in cooperation with the Forest Products Laboratory to obtain information on the species and distribution of blue-staining fungi infecting the sapwood of hardwoods and of softwoods in the continental United States and in Canada. These fungi appear to divide themselves into three general groups which are found respectively in three different places: In the forest, on green wood in the lumber yard, and on wood that is old or that has been heated.

FUNGI IN THE FOREST

Although Ceratostomella and Graphium appear to be the most common of the staining fungi in every part of the country, they are found in the greatest variety of forms in the forests. Here also are some Pyrenomycetes, pycnidial forms, perhaps of Valsa, and some molds, many of them non-staining, among which Trichoderma is important.

FUNGI ON GREEN LUMBER

The flora developing in the lumber yard on the freshly sawed boards seasoning in the stacks is less varied than that in the forest. Ceratostomella and Graphium are still the fungi commonly found, but the number of forms has decreased. *Ceratostomella pilifera,* the blue-staining fungus common in lumber yards in all parts of the United States, is the important form; *Graphium rigidum,* the gray or bluish gray-staining one, is frequently a companion of it. These fungi are hardy and rapid growers. Other varieties of Ceratostomella and Graphium also occur on such lumber; and, in addition, there are some fungi of the Valsa-like group and some molds, but they are not the important stainers of the freshly cut and seasoning boards.

FUNGI ON LUMBER THAT HAS BEEN HEATED OR DRIED

After the sapwood boards have been steamed or kiln dried, or when they are old, the flora living in the blue-stained wood is still different. It consists now of the Valsa relatives, molds, and a black mycelium, which has not yet fruited in culture. Ceratostomella and Graphium do not infect such boards. Wood rots generally are present in the old wood, but they do not stain the wood blue. Also most of the molds found have colorless mycelium instead of brown and probably do not stain.

TABLE 1.—*Fungi cultivated from blue-stained sapwood collected in the United States and Canada*

Host genus	Number of States and Provinces represented	Total number of specimens examined	Number of specimens that yielded Ceratostomella (C) or Graphium (G)											Number of specimens yielding				Other fungi
			1C	2C	3C	1G	1C 1G	2C 1G	1C 2G	3C 1G	2C 2G	4C 1G	3C 2G	*Valsa* sp. (pycnidial stage)	Sterile black fungus	*Trichoderma lignorum*	*Endoconidiophora* sp.	
Fresh specimens from the forest																		
Quercus	4	31	1	2	1	3	8	6	1	2	1	1		3	1			Atractium, Stemonites, Pestalozzia
Pinus	5	23	13	1		3	1							1			3	*Dematium pullulens*
Liriodendron	3	9	1	1	1	2		3		1								
Hicoria	5	5			1		2			1			1					
Liquidambar	2	5		1	1		2	1										
Fagus	2	4					1	2		1								
Aesculus	2	2			1					1								
Tilia	2	2	1					1										
Tsuga	1	2				1	1											
Bignonia	1	1												1				
Castanea	1	2					1	1										
Gleditsia	1	1						1						1				
Populus	1	1							1									
Prunus	1	1					1											
Specimens from stacks of green lumber in yards																		
Pinus	6	23	13			3	3								3			Stachybotrys
Picea	3	6				6												
Liquidambar	2	4	1			1	1									1	1	
Magnolia	1	4	3				1								1			
Tilia	1	4												2	2			
Tsuga	1	3				3												
Betula	1	2				1	1								1			
Quercus	1	1												2 spp.				
Acer	1	1	1															
Specimens of heated and of old wood																		
Pinus	6	10												1	1	2		3 Wood rots,[1] Stemonitis, *Penicillium pinopholum*
Liquidambar	2	5												2	2	1		Alternaria, *Monilia sitophila*, 2 Penicillium
Quercus	1	1													1			

[1] These wood-rotting fungi were in the blue-stained wood of logs that had been lying in the forest 3 years. It is probable the blue-staining fungi had died.

OBSERVATIONS

Table 1 shows the general situation. The fungi listed were growing in blue- or gray-stained sapwood; an explanation of how they were cultivated from the wood will make the data in the table clearer. First, they were obtained by gouging out a sliver of the discolored wood from the interior of the specimen with a quarter-inch-wide forceps-chisel. The sliver was always taken from the inside of the board, chip, or branch, in order to avoid picking up fungus spores that perchance might have fallen on the surface of the specimen without being implicated in its stained condition. After being thus cut out the slivers were transferred to malt-agar slants, where they had an opportunity to grow from the wood to the agar. Naturally, only such fungi were cultivated as were vigorous enough to grow out on the agar. Doubtless many species and numerous varieties were suppressed by more vigorous ones and hence were overlooked when retransfers were made. Still, it is a surprising fact that as many as three strains of Ceratostomella and two of Graphium have grown from a sliver of wood, no more than a quarter-inch wide, taken from a chip picked up in the forest.

"Ceratostomella and Graphium" in the heading of the fourth column of the table means that Ceratostomella and Graphium are separate fungi. In other words, the Graphiums in general are not imperfect stages of Ceratostomella.

As a rule, the specimens from the forest were infected with more than one strain of fungus. The pines were exceptions to this rule except in two cases. One of the reasons for this is the fact that 7 of the 23 specimens of pine listed had been taken from trees infested with the bark beetle Dendroctonus and 8 with the beetle Ips. Ordinarily, the freshly bored canals of these beetles are infected with only a single blue-stain fungus, specific for Dendroctonus, or, another, specific for Ips. In the case of one of the pine specimens infested with Dendroctonus, however, the brood of beetles had died in the trees. When cultured the blue-stained wood from this specimen produced *Ceratostomella pilifera* besides the fungus commonly associated with the bark borer. The other example of a condition unusual for the forest pines examined was a specimen infested with Ips, which produced *Graphium penicillioides* in addition to the Ceratostomella found with Ips.

OFFICE OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

ANOTHER ANTHRACNOSE OF RASPBERRY

S. M. Zeller

During the last five years the writer has collected from various places in western Oregon and Washington samples of a Gloeosporium which produces an anthracnose of the wild raspberry (*Rubus leucodermis* Douglas), in particular. Rather infrequently the disease has been found on cultivated varieties of black raspberry and the St. Regis variety of red raspberry. So far as has been observed this occurs only when the cultivated varieties have been planted near infected *R. leucodermis* in neighboring wood-lots. The known distribution of the disease is from Douglas County, Oregon, to British Columbia, but perhaps it is more widely distributed over the range of the wild host, *R. leucodermis*.

The causal organism answers in every morphological character the description of *Gloeosporium allantosporum* Fautrey. Fautrey says this species differs from *G. vincetoxici* Fautrey in spore size, but otherwise is similar except in host preference. Perhaps this is a variety of the latter species but we prefer to refer to it as *G. allantosporum*. The name is applicable to Pacific Coast material, the spores of which are characteristically allantoid and measure 16–20 x 3 μ.

DESCRIPTION OF LESIONS

So far as known the disease is confined to the canes. The spots on the canes are long and narrowly elliptical. First infections of new canes take place with the first rains in the fall, but later infection may occur well into the spring. The lesions first appear as longitudinal marks, about ¼-inch in length and as broad as ordinary pencil marks. These long, narrow early indications of infection in the fall are greenish-brown with a water-soaked appearance. These enlarge very slowly and become pale-yellowish and then as the canes become dormant in the late fall, the lesions turn white and ashen. The lesions are not so deeply sunken as in the case of the well-known anthracnose of black raspberries caused by *Plectodiscella veneta* Burk., due perhaps to the fact that the vascular and more firmly organized elements of the bark of the host are rather well matured before infection takes place. Infection involves the epidermis and outer layers of cortex only. Lesion margins become reddish brown, forming a considerable "halo" around the lesions in most cases. The mature lesions are long and narrow in contrast to the very nearly circular ovals in the anthracnose caused by *P. veneta*. Lesions produced by the latter often occur on the same canes along with those produced by *Gloeosporium allantosporum*.

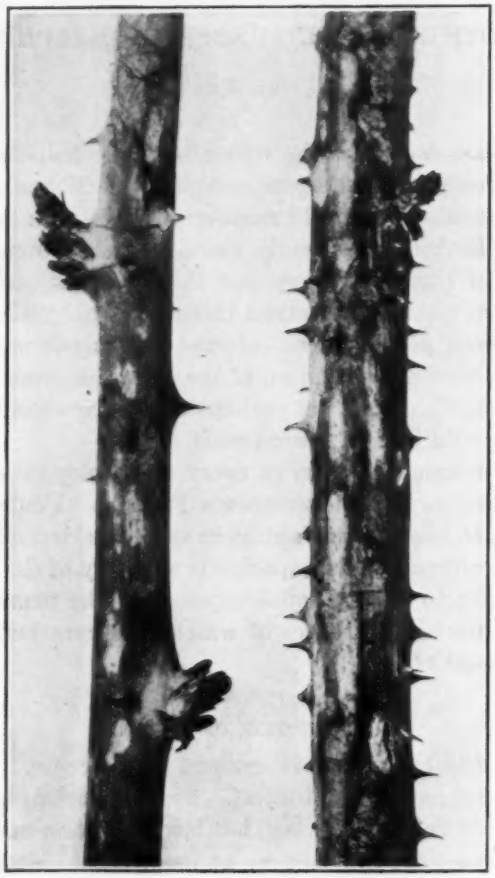


FIG. 1. Lesions produced by *Glocosporium allantosporum* on canes of *Rubus leucodermis*. The definite, elliptical lesion on the lower part of the right-hand cane is produced by *Plectodiscella veneta*.

The lesions do not show any indications of fruiting bodies the first season, but early in the spring from February until June and again in the fall, the acervuli appear as tiny black dots over the surface. The cane lesions do not materially differ in appearance from those produced by *Rhabdospora rubi* Ellis.[1] The acervuli are often so close together that the canes are practically girdled. In such cases, however, the disease is not so serious as in the well-known anthracnose because the lesions are not so deep-seated but extend only a short distance under the epidermis.

[1] Zeller, S. M. Contribution to our knowledge of Oregon fungi. Mycologia 21: 97–111. 1929. (See Fig. 2, p. 109.)

CONTROL MEASURES

In the fall of 1926 and spring of 1927, it was convenient to apply sprays to some of the western wild raspberries in a locality where this anthracnose was very bad. These two sets of spraying tests were applied in the following manner. In 1926 in the first trials Bordeaux 4–4–50 was applied before the fall rains began and in the spring of 1927 in the second spray test two applications of Bordeaux 4–4–50 were given, one when the canes were 18 inches high and another when about 25 inches high. In the first test the plants so sprayed had no anthracnose in the spring of 1927. In the case where one application was applied when the canes were 18 inches high and again when 25 inches high there were present in the spring of 1928 eighty-seven per cent as many lesions caused by *G. allantosporum* as on neighboring plants which were left unsprayed. In these cases, however, the lower 18 inches of the canes had decidedly fewer lesions than the portion higher up. The spray applied just before the discharge of spores in the fall is effective in controlling new infections on the canes grown during the current season. This information will perhaps help in the control of this disease in black raspberry plantings near open wood-lots where infected plants of wild black raspberry are present.

SUMMARY

In western Oregon and Washington, an anthracnose caused by *Gloeosporium allantosporum* occurs on the wild black raspberry (*Rubus leucodermis*) and on cultivated varieties of black raspberry and the St. Regis variety of red raspberry when the latter are planted near the wild host harboring the disease. This paper describes the disease briefly. Bordeaux 4–4–50 applied just before the discharge of spores in the fall controls the disease on canes grown during the current season.

OREGON AGRICULTURAL COLLEGE,
EXPERIMENT STATION,
CORVALLIS, OREG.

PHYTOPATHOLOGICAL NOTES

A mutation in Gloeosporium. While a number of petri-dish cultures of Gloeosporium, isolated from a lemon, were being grown in the Mycology Laboratory, one culture behaved differently from the rest. Instead of producing the usual dark appearance (Fig. 1, 1) due to an abundance of acervuli, it had in parts the characteristic dark appearance (Fig. 1, 2A)

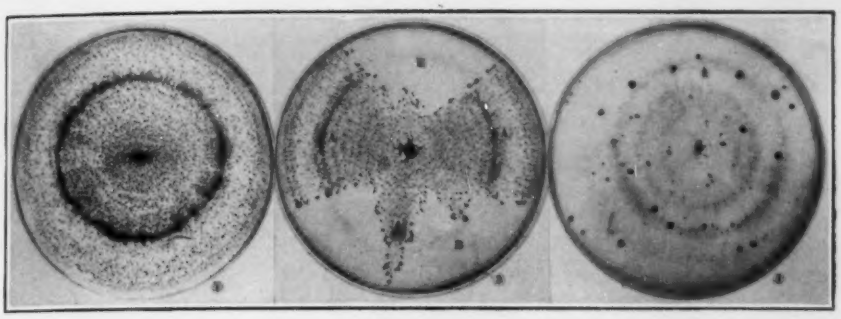


FIG. 1. 1. Gloeosporium culture from 2A, the usual appearance.
2. Culture from a plate similar to that in 1, showing the usual type of growth in A and the new form in B.
3. Culture from 2B showing the new form of growth as contrasted with the old form in 1.

and in others a lighter appearance (Fig. 1, 2B) due to the presence of only a few scattered orange and black acervuli. Subsequent cultures made from the dark (A) and light (B) segments showed that the respective transfers remained constant for the characteristics present in the original segments (Fig. 1, 1 and 3). It would appear then that a sudden and permanent variation or mutation had occurred. No variation was observed in conidia form.—JACQ. P. F. SELLSCHOP, Department of Botany, University of Illinois, Urbana, Ill.

A note on the longevity of the bean mosaic virus. A collection of 150 varieties of domestic and foreign beans made in 1897 by the Horticulturist of the Idaho Agricultural Experiment Station was recently turned over to the Department of Plant Pathology. These varieties were stored in 6″ glass vials and bore the date 1897. Horticultural records show that they were planted in 1898 and 1899, but there is no evidence of their having been planted since that time. In attempts to germinate seed of a large number of these varieties, only the following were viable: New German Wax Flag; Guatemala Red (native variety); a strain of Frijol bayo from Matamoras, Mexico; and an unnamed black wax variety, No. 24.

On February 20, 1929, from 10 to 25 seeds of each of the above varieties were planted in the greenhouse. With the exception of the black wax

variety (No. 24), all of the plants obtained were apparently free from disease. Only two black wax plants were secured, but one of these exhibited mosaic mottling in the two seed leaves as soon as the plant emerged. As new leaves appeared, unmistakable mosaic mottling also could be seen in them. There was no curling of the leaf margins associated with the mottling, but the leaves were considerably narrower and longer than those of the healthy plant.

On March 22, several of the mottled leaves were removed from the plant and thoroughly macerated in a sterile mortar. This material then was used to inoculate 12 healthy Great Northern bean plants. Symptoms of mosaic typical of the Great Northern variety had appeared on six of the plants by the 3rd of April. A few days later, 10 plants showed mosaic symptoms, and thus furnished conclusive evidence that the bean mosaic virus had remained viable within the seed for a period of at least thirty years.—W. H. PIERCE and C. W. HUNGERFORD, Department of Plant Pathology, University of Idaho, Moscow, Idaho.

Summer meetings of the Botanical Society of America. In accordance with the sentiment expressed by the members in a recent poll, The Botanical Society of America will conduct two summer meetings during the present year. One of these will be held at Dartmouth College, Hanover, N. H., beginning on the evening of June 25 and extending through June 28. The other will be held at the mountain camp of the University of Wyoming from July 31 to August 3.

These meetings are not to be patterned after the regular winter meetings, where emphasis is placed upon the presentation of the results of research in formal papers. They will be characterized rather by informality, the time being devoted to field excursions and round-table discussions. Everything will be done to promote that personal association out-of-doors which is necessarily lacking at the more formal and crowded meetings.

At the Dartmouth meeting it is planned to have an informal reception and an address of general interest on the opening evening, a number of morning round-table discussions of special topics, two afternoon field trips, one all-day excursion, and an open discussion of botanical teaching methods.

Registration for the Wyoming meeting will occur at Laramie on the afternoon of July 31; this will be followed by an informal dinner and reception. On the following morning those in attendance will be taken by automobile to the mountain camp, where three days will be occupied by field excursions and round-table discussions.

The Chairman of the Pacific Section of the Society has invited the members to consider the annual meeting of the Section, to be held in Berkeley on June 20 and 21, as another summer meeting of the Society.

A cordial invitation to attend the meetings is extended to botanists who are not members of the Society, and especially to those who may be visiting this country from abroad.

A circular containing more detailed information was mailed to every member of the Society in May. Inquiries may be addressed to the undersigned or to the chairmen of the local committees: Prof. Aven Nelson, University of Wyoming, Laramie, Wyoming, and Prof. A. H. Chivers, Dartmouth College, Hanover, N. H.—L. W. SHARP, *Chairman of the Organizing Committee*, Cornell University, Ithaca, N. Y.

INTERNATIONAL CONFERENCE FOR PHYTOPATHOLOGY AND ECONOMIC ENTOMOLOGY—HOLLAND, 1929

SECOND NOTICE ON ERIKSSON PRIZES

The International Committee for Phytopathology and Economic Entomology beg to announce that two prizes are hereby offered for the two best Memoirs, giving an account of new and original work on the two following subjects respectively:

(1) Investigations on Rust (Uredineae) Diseases of Cereals (Wheat, Oats, Barley or Rye).

(2) Investigations on the rôle played by insects or other invertebrates in the transmission or initiation of Virus Disease in Plants.

The value of each prize will be 1,000 Swedish crowns.

Competitors may be of any nationality.

Three typewritten copies of each Memoir must be submitted. They may be written in any one of the three languages, English, French or German.

Memoirs must reach the Secretary on or before May 1st, 1930.

The author's name must not appear on the Memoir itself, but each Memoir must be marked with a pseudonym or a motto and the full name and address of the author must accompany the Memoir, being enclosed in a sealed envelope bearing on its outside the same pseudonym or motto as is given on the Memoir.

The adjudication of the rust prize will rest with a jury, consisting of Professor Dr. Jacob Eriksson, Professor Dr. E. C. Stakman and Professor Et. Foëx.

The adjudication of the virus prize will rest with a jury, consisting of Professor Dr. H. M. Quanjer, Dr. A. D. Imms and Dr. L. O. Kunkel.

The decisions of these juries will be final and will be announced at the Fifth International Botanical Congress, to be held in Cambridge (England) from August 16th to 30th, 1930. The copyright of the prize Memoirs will become the property of the Committee, who will endeavor to secure publication of them in a suitable existing periodical or, failing that, procure

publication in some other way. Other Memoirs will be returned to their respective authors.

The Committee reserve the right to withhold the prizes should none of the Memoirs submitted be deemed of sufficient merit by the respective juries.

Further particulars, if required, may be obtained on application to the Secretary.

For the International Committee for Phytopathology and Economic Entomology.

T. A. C. SCHOEVERS, *Secretary*,
Nassauweg 28, Wageningen, Holland.

Notice of Summer Meeting. The summer meeting of the American Phytopathological Society will be held in conjunction with the regular annual meeting of the Southern Division, July 28–31, 1929. The committee in charge: R. F. Poole, Chairman; G. W. Fant, Secretary; S. G. Lehman, S. A. Wingard, and G. M. Armstrong, has arranged a three days' meeting and tour starting at the College of Agriculture at Raleigh, thence northward to Oxford, then southward to Duke University at Durham, the University of North Carolina at Chapel Hill, Sanford, Southern Pines, Pinehurst, and Hamlet. This itinerary will take the group through some of the important educational centers and famous winter resorts as well as through some of the most intensive and interesting crop sections. Ample opportunity will be given to observe and study diseases of crops such as tobacco, sweet potatoes, cotton, peach, soybeans, and dewberries. The cooperation of the horticulturists and agronomists of the Agricultural College has been obtained. Demonstrations and experiments on disease control have been arranged at various points along the line of travel. These attractions, coupled with the fact that the spring and early summer weather has been favorable for plant-disease development, should insure a successful meeting.

Those who expect to attend should communicate with the chairman of the committee at the North Carolina State College, Raleigh, as soon as possible.—COMMITTEE ON ARRANGEMENTS.

Lyman Memorial Fund. The total of the Lyman Memorial Fund for phytopathological publications already is about $1,000.00. This includes cash on hand and pledges. Individual cash donations range from $1.00 to $50.00, and the pledges range from $1.00 a year for a short period of time to $25.00 a year for an indefinite period. It appears that the project will be a success. It is hoped that the endowment fund will continue to grow.

In addition to the Lyman Memorial Fund, the National Research Council has generously given $500.00 to be used before July 1, 1929.

BOOK REVIEW

Handbook of Microscopical Technique. For workers in both animal and plant tissues. Consisting of contributions from 24 specialists, edited by C. E. McClung. *XIV + 495 pp. 43 figs.* Price $8.00. Paul B. Hoeber, Inc.: New York, 1929.—The following statement is from the preface: "In two previous texts, General Cytology and Special Cytology [ed. by E. V. Cowdry] experts in various fields of biology have presented the results of their investigations. In the present work, which extends the series, the *methods* involved in these and similar studies are given. There are two general needs to be met by a work of this sort. The first is that of the unexperienced worker who requires specific directions which he may apply with confidence to the general run of material, with the expectation of getting desired results. (Part I . . . outlines such standard methods.) The other requirement is that of the experienced investigator who seeks the latest approved methods for the accomplishment of special technical results. In order to meet such a need as this, involving, as it does, the intimate knowledge of varied and often involved processes, the method of cooperation employed in the above-mentioned books is utilized. Workers highly experienced in the use of specialized methods describe this in detail."

A partial list of the chapter headings and the respective authors is: Section and non-section methods of preparing microscopical slides, C. E. McClung; Microdissection, microinjection, Robert Chambers; Vital stains, Nathan Chandler Foote; Supravital stains, Florence R. Sabin; Bacteriological methods, H. J. Conn, F. B. Mallory, and Frederic Parker, Jr.; General botanical microtechnique, William Randolph Taylor; Protozoological methods, D. H. Wenrich; Fixation and fixatives, C. E. McClung and Ezra Allen; Stains and staining, C. E. McClung and H. J. Conn.

The remaining chapters have special reference to zoological and medical cytology and histology, though among the many details presented doubtless much of value to the special worker in other fields also is to be found.

The discussion of non-section methods, though written from the viewpoint of a zoologist, should be of considerable value to workers in plant tissues, among whom a tendency to rely perhaps too much on sectioning methods has been manifest. Of special importance is the discussion of methods for the study of fresh material, including microdissection, microinjection, vital staining and intracellular indicators—in short the "newer" technique. Certain of these methods are already in use in special studies of plant cells as physical properties of cell constituents, nature of inclusions; others can be adapted.

In the chapter on botanical microtechnique, the treatment of special subjects, such as fungi and the differentiation of fungus mycelium from

host tissue, is somewhat less extensive than in a book particularly devoted to the botanical field, as Chamberlain's Methods in Plant Histology, but phytopathologists might wish for more emphasis on this subject in either work.

Throughout the book particular emphasis is placed on the rationale of technical methods. This feature should further promote the proper application of the details and authoritative exposition of processes.—FREEMAN WEISS, Bureau of Plant Industry, Washington, D. C.